

XXXXXXXXXXXXXXXXXXXXXXXXXXXX

March 14, 1973

Subject: Screwworm Nutrition

To: H C Cox, Assoc. Deputy Administrator
USDA-ARS, Southern Region
New Orleans, La. 70153

Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Invest.

On Monday, March 12th, I talked with Dr. Bushland about reviewing the nutritional requirements of the screwworm.

The following plans have been made. I will go to Mission, Texas on Thursday, ~~Mar~~ 29, 1973. On Friday I will tour the lab and become familiar with the operation. The weekend will be spent in reading and study of any material that Dr. Bushland or his staff suggest. Dr. R. E. Gingrich will come to Mission for consultation on Monday, April 2 and remain through Tuesday. I will plan to return to Florence as soon as possible after this but will be able to stay in Mission through Friday, April 6 if necessary.

I appreciate the opportunity to participate in this program. I don't know if I can render any assistance but will make every effort to be useful.

R. F. Moore, Jr.
Research Entomologist

cc:

W. M. Bruce
R. C. Bushland
W. Klassen
R. E. Gingrich

REFERENCE SLIP

3/19/73

TO

R. C. Bushland

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ACTION

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NOTE AND RETURN

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APPROVAL

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PER PHONE CALL

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AS REQUESTED

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RECOMMENDATION

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REMARKS

FROM

Edgar A Taylor

2/19/73

ENTERING SLIP

R. C. Bushland

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John A. Taylor

MAR 14 1973
Wesley

copy to Bushland +

21 MAR 1973
MAR 9 1973
[Signature]

March 6, 1973

SUBJECT: Screwworm Nutrition

TO: Dr. J. Rex Johnston, Acting Area Director
ARS, USDA, Oklahoma-Texas Area, Southern Region
Bushland, Texas 79012

THROUGH: Dr. R. O. Drummond

As you know from the memorandum of February 26 from Dr. Waldemar Klassen, I have been asked to review the present problem with the performance of screwworm flies in the eradication program. I have cooperated in the past with the personnel at the rearing plant in Mission on nutritional problems and would be pleased to do so again.

When consulted before on screwworm problems, I encountered situations similar to the present one. Namely, there is doubt about the performance in the field of laboratory reared flies. Among the many possible causes for the poor performance it has been suggested that the flies had low vitality - a condition possibly related to their diet and other rearing procedures. Also, as before, there still seems to be difficulty in delineating the problem. Without this ability you cannot assign the problem to nutritional factors. If you assume this is the fault you still lack the specific goals needed for experimentation and methods for the field evaluation of your results.

Previously I was consulted when it was decided that the problem was the inferior size of artificially reared flies. Our goal of rearing larger flies was achieved but there was no way to determine if this was in fact the solution. Later, after further difficulties with eradication efforts occurred, it was suggested by some that maybe our flies were too big--perhaps they were just big and lazy but at least there was no way to test to settle the question one way or another.

I see the same problems with the present situation. Poor longevity of the presently used (old) strain is mentioned, but since the new strain is living longer on apparently the same diet I don't think nutritional factors are to blame. This is possibly a genetic difference. Robustness was also mentioned, but again this is a nebulous goal and one that will need testing in the field.

March 6, 1973

Dr. J. Rex Johnston

Page 2

I think the suggestion of Dr. Klassen that comparative chemical analyses of wound-reared and artificially reared screwworms is sound and would possibly provide valuable background data, although I am not convinced that rearing an insect with a similar chemical composition to a wound-reared individual would insure the proper performance of the product. There is much data available on insect nutrition that can be applied now to manipulate and alter the final screwworm product, but the basic problem remains - what characteristics in a fly do you aim for and how do you field test to determine if this characteristic enables the fly to do the job you want.

I have not been closely associated with the screwworm eradication program the past 2 years and I have not been informed of recent developments. If the problems I mentioned above no longer exist then I believe there are immediate positive goals that can be achieved through further nutritional studies. In any case, I am willing to lend whatever assistance I can in further cooperation with the program.

Richard E. Gingrich
Research Entomologist

cc:

W. Klassen

R. F. Moore

E. A. Taylor ✓

W. M. Bruce

ARS:RERgingrich:jf (3-6-73)

REFERENCE SLIP

3/23/73

TO

P. C. Bushland

28 MAR 1973

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YOUR SIGNATURE

REMARKS

This is 1st draft
of the write up for
other work done at
Mission. We thank
you for your cooperation.

RAT

FROM

J. C. Brinkley

3/22/73

- | | |
|---|---|
| ☐ DATE AND TIME | ☐ NAME |
| ☐ THE PHONE CALL | ☐ ADDRESS |
| ☐ INFORMATION | ☐ COMMENTS |
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| ☐ FOR SIGNATURE | ☐ SIGNATURE |

He is 1 1/2 days
of the work done at
Manning. We thank
you for your cooperation.

PAH

For: J. Med. Entomol.

Please send proof to:

James E. Wright

P. O. Drawer GE

College Station, Texas 77840

HORMONES FOR THE CONTROL OF LIVESTOCK ARTHROPODS.

EFFECTS OF 2 JUVENILE HORMONE ANALOGUES AGAINST THE SCREWORM,

COCHLIOMYIA HOMINIVORAX (COQUEREL) AND INFESTED BOVINE HOSTS^{1/}

by

James E. Wright, H. E. Smalley, R. L. Younger, and

H. R. Crookshank^{2/}

Veterinary Toxicology and Entomology Research Laboratory

USDA, ARS, P. O. Drawer GE

College Station, Texas 77840

ABSTRACT: Two juvenile hormone analogues, 6,7-epoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene and isopropyl 11-methoxy-3,7,11-trimethyldodeca-2,4-dienoate inhibited the emergence of the screwworm, Cochliomyia hominivorax (Coquerel) when applied topically to mature larvae and pupae and when incorporated within the larval diet. Two bovines that were sprayed with a 0.1% concentration of ^{each} analogue and then infested with larvae of the screwworms displayed no changes in serum chemistry and adults eclosed from the wound-reared larvae.

Juvenile hormone (JH) active analogues do not have a broad spectrum activity against insects of different orders but tend to be highly specific in action toward individual species (Roller & Dahm 1968, Slama 1971). Thus, the JH's tend to have greater possibilities of selected activity against species within the same order. For example, analogues that are active against the stable fly, Stomoxys calcitrans (L.) and the house fly, Musca domestica L. tend not to be active against the yellow mealworm, Tenebrio molitor L. or the large milkweed bug, Oncopeltus fasciatus (Dallas) or vice versa (Jacobson et al. 1972, Wright & Sonnet 1973).

Laboratory and field studies have demonstrated that the Stauffer R-20458, 6,7-epoxy-1-(p-ethylphenoxy)-3,7-dimethyl-2-octene, is active against the stable fly but not against the house fly (Wright 1972, Wright & Spates 1972, Wright et al. 1973). The Zoecon 515, isopropyl 11-methoxy-3,7,11-trimethyldodeca-2, 4-dienoate, is active against both the stable fly and house fly in the laboratory but is inactive when applied to natural breeding areas in cattle feedlots (Wright et al. 1973). Both analogues are active also against several species of mosquitoes (Jacob 1972, Schaefer and Wilder 1972). Since the activity of these two JH analogues is primarily concerned with dipteran species, this study was undertaken to define activity within one of the more important North American dipteran species, the screwworm, Cochliomyia hominivorax (Coquerel). Eradication of this pest species from the U. S. was virtually complete until 1972 and then the largest number of screwworm cases occurred since the eradication program had begun. Thus, the opportunity for

further substantiation of the JH activity of compounds active on a species within a given order was extended to include the screwworm with these two active analogues.

MATERIALS AND METHODS

The R-20458 was furnished as a 50% emulsifiable concentrate (EC) with AI of 44.1% trans isomer as determined by Bowman et al. (1973). A technical sample of 74.9% trans isomer was used in a solution of acetone (Spectranalyzed® grade) for topical treatments. Both were supplied by Stauffer Chemical Co., Mountain View, Calif.

The ZR-515 was supplied by Zoecon Corp., Palo Alto, Calif., as an EC with an AI of 52.5% trans isomer, as a technical sample of 68.9% trans isomer, and as a slow-release preparation containing a 10% trans isomer (SR-10).

The two JH analogues were evaluated by making topical applications to mature third instar larvae and to pharate pupae and pharate adults within the puparia and by placing the analogues in the larval rearing media.

Larvae were collected immediately after crawling from the rearing vats and 3 samples of 25 were treated topically with 2 ul of concentrations of 10, 1.0, 0.1, .01, .001, and .0001% solutions. At 3 and 8 hrs later another series of larvae were treated. At 24 hrs, pupation had occurred, a series was treated and another subsequently at 48 and 72 hrs. At 24 hrs, 3 samples of 25 pupae each were dipped for 10 seconds in the technical R-20458, ZR-515 and SR-10. All test insects were held at 25°C

and ca. 85% RH and observed for emergence after 14 days. Those puparia from which no adults eclosed were dissected to determine if pupal-adult intermediates similar to those of the stable fly, Stomoxys calcitrans (L.) (Wright 1970) or the fleshfly, Sarcophaga bullata Parker (Srivastava & Gilbert 1969) were present.

SR-10 and EC's of R-20458 and ZR-515 were incorporated into the larval diet. The first 24 hours larvae (ca. 100) fed on a media composed of suckle (dried milk), dried egg, horse meat, and formalin (total mixture of 400 g). The analogues were incorporated into the diet at the rates of 10,000, 1000, 100, 10, and 1 ppm. Each day thereafter for 5 days, new diet (400 g) was prepared with the analogues added and the larvae were removed from the old to the freshly prepared diet. The larvae were allowed to crawl from the treated media into sawdust where pupation occurred and then checked for eclosion 14 days later. Those puparia from which adults had not eclosed were dissected as previously stated.

Two yearling heifers, ca. 220 kg each, were sprayed with 0.1% concentration (3 gal/animal) of IGR I and IGR II and infested with newly-hatched larvae in artificial wounds on the hips at 2 and 10 days after spraying. Larval development, wound healing and serum chemistry of the animals were observed. Blood was collected from the jugular vein prior to treatment and then weekly for 4 consecutive weeks. The wound-reared larvae were collected as they left the wound and held for eclosion.

Serum phosphorus, uric acid, urea, nitrogen, creatinine, glucose, and total serum protein were determined with a single channel Technicon

Auto-Analyzer® using the N-chemical methods. Serum calcium, magnesium, potassium, sodium, zinc, copper, and iron were determined with a Model 403 Atomic Absorption Spectrophotometer as outlined in the Perkin-Elmer manual.

The serum proteins including albumin, α , β , and α -globulins were determined electrophoretically using the Beckman Microzone, cellulose acetate electrophoresis patterns and the Beckman Model R-111 Microzone Computing Densitometer System. Serum amino acids were studied on a Beckman Model 121 Automatic Amino Acid Analyzer with an Infratronics Automatic Digital Integrator, Model CRS-210. Beckman methods as found in the Beckman Instrument Manual for this model analyzer were used, using resin PA-28 and PA-35. Cholinesterase inhibition was studied using a whole-blood modification of the Michel Method. Additionally, information was collected on clot retraction, platelets, and fibrinolysis.

RESULTS AND DISCUSSION

The results of the topical applications to larvae and pupae are given in Table 1. This data tend to confirm that JH compounds that are highly active against specific species of the same order will be active against other species within that order (Wright and Beroza, in press). The screwworm has a period of sensitivity to the JH analogue ZR-515 which extends to 48 hrs after cessation of larval feeding and for R-20458 a period probably not exceeding 72 hrs. This length of time is somewhat surprising in that the sensitive period for other insects is considerably shorter than this, i.e., the last third of the last larvae stage of Galleria mellonella (L.) (Sehnal and Meyer 1968),

mature larvae of Aedes aegypti (L.) (Spielman and Skaff 1967), and the white pupal stage of the stable fly (Wright 1970). Dissection of the puparium from which no adult had emerged revealed a pupal-adult intermediate, i.e., the head and thorax had developed adult characteristics whereas the abdomen was morphologically that of a pharate pupae. No adults eclosed in the test when larvae were earlier dipped in the technical solutions of R-20458 and ZR-515 and SR-10.

In the larval diet treatments, all larvae were dead in the R-20458 and ZR-515 tests at 10,000 ppm after 24 hrs. The SR-10 test had small larvae that had no indication of growth. The 1000 ppm treatment of R-20458 and ZR-515 had a few live larvae with those in R-20458 showing no signs of growth whereas those larvae in ZR-515 were of various sizes from first to second instars. No effects were observed in the lower treatments at 24 hrs. At 48 hrs the remaining larvae were dead in the 1000 ppm of R-20458. In the SR-10 at 1,000 ppm the larvae were alive but showed no apparent growth. Pupae were obtained from R-20458 and ZR-515 in the 100, 10, and 1 ppm treatments. However, no adults emerged from the R-20458 treatments whereas adults emerged from the 1 ppm ZR-515 and 10 and 1 ppm of SR-10. In all 3 treatments larviform pupae and dead third instar larvae were present within the treated media.

The developmental time of the screwworms in the Angus heifer treated with ZR-515 exceeded that of the screwworms in the Hereford heifer treated with R-20458 in the wounds made 2 days after spraying. Larvae were still present in the wound at 8 days post infestation of the ZR-515

animal whereas all larvae had left the wound by 5 days in the R-20458 animal. However, no noticeable difference occurred in the larval developmental time in the second wound and infestation made at 10 days posttreatment. All larvae had left the wounds in both animals 5 days post infestation. The first wound in the R-20458 treated animal healed rapidly whereas the first wound in the ZR-515 treated animal was slow to scab over and appeared reddish, wet and weepy. Both wounds attributable to the second infestation healed without any noticeable differences. Adults emerged from all the larvae that were reared in the wounds.

While there were fluctuations in the levels of the serum minerals and nitrogen components measured, the changes were all within "normal" levels. The serum protein electrophoresis patterns did not indicate any significant changes. Serum magnesium, sodium and zinc decreased during the test period while copper increased. Serum calcium increased slightly in the R-20458 treated animal during the test whereas the ZR-515 treated animal did not show any change. Both animals had a decrease in serum urea nitrogen through week 3 and returned to the initial level by week 4. Only minor fluctuations were observed in the protein electrophoretic patterns in both quantity and relative percent of the albumin and globulin fractions.

However, the data for the amino acids of the 2 treated animals (Table 2) suggest strongly that the JH treatments caused a decrease in the amino acid levels the first week after treatment and then the levels gradually ascended to the pretreatment levels.

The conclusion from measuring the different parameters in the JH treated animals is that the systems identified responded little or any except the amino acids, to the JH and this in turn did not affect the development of the screwworm larvae. The immediate decrease in the amino acids after JH application could possibly be an unexplainable toxicological manifestation of the analogues. This particular phenomenon was also noted in a separate test utilizing sheep and R-20458, i.e., an immediate decrease followed by return to normal values with no signs of toxicity apparent (Smalley, unpublished data). Certainly, from comparing the sensitivity of the screwworm in the bioassays, the parent compounds were not present in the animals at levels to affect the developing larvae. Only in the first infestation in the ZR-515 treated animal was any indication of growth retardation and then only a few larvae had not matured within the usual time period of 5 days.

Because of differences observed in the clotting behavior of the samples drawn for serum chemistry, we did a fibrinolysis screening test that is based on the principle that an excess of fibrinolytic activity present in the blood will impair the formation of the clot which will quickly lyse after incubation at 37°C (Miale 1974). Lysis began in the serum from the ZR-515 animal in 6.9 minutes whereas in the R-20458 animal lysis began in 9 minutes. A mixture of equal parts plasma from each animal began lysing at 6.4 minutes. We concluded that the plasma from ZR-515 animal contained an abnormal amount of fibrinolytic material. This was further substantiated by observations of clot retraction mechanisms in both animals and may account for retarded healing time noted in the early infestation of the ZR-515 animal.

FOOTNOTES

1/ This paper reflects the results of research only. Mention of a proprietary product or a pesticide does not constitute endorsement or recommendation by the USDA.

2/ This research was done at the USDA-ARS laboratory at Mission, Texas in cooperation with Dr. R. C. Bushland and with the assistance of Dr. M. M. Crystal, Bob Handke and Joe Garcia of that laboratory and Ms. E. Steel, J. Cooper and F. Farr of the College Station Laboratory.

TABLE 1. Sensitivity of screwworm larvae and pupae to R-20458 and ZR-515 applied topically.

Treatment time (hrs) ^{a/}	% inhibition of adult eclosion caused by indicated concentration of R-20458 and ZR-515 ug/insect					
	200	20	2	0.2	.02	.002
	<u>R-20458</u>					
1	88	100	100	100	100	84
3	84	100	100	100	100	88
8	100	96	100	100	100	100
24	96	92	92	100	100	100
48	84	100	100	100	96	100
72	92	92	88	100	56	36
	<u>ZR-515</u>					
1	88	88	100	96	92	100
3	100	100	100	76	92	96
8	100	100	100	80	96	92
24	100	100	100	96	92	88
48	100	100	100	100	96	96
72	20	28	20	16	20	24

^{a/} 1, 3, and 8 hr were larval stages of that age and 24, 48 and 72 were pupal stages of that age.

TABLE 2. Serum amino acids of the 2 JH treated animals.

Amino Acid	$\mu\text{m}/100\text{ ml}$									
	R-20458					ZR-515				
	Date serum collected									
	1-16	1-23	2-1	2-8	2-15	1-16	1-23	2-1	2-8	2-15
Phosphosprine	<u>1</u> /	-	-	-	-	-	-	-	-	-
Phosphoethanolami ^e	1.2	-	2.7	0.9	0.5	0.9	0.8	0.4	0.7	0.7
Taurine	7.5	7.5	11.0	8.0	8.8	4.4	2.8	5.0	7.6	7.6
Urea	578.4	279.7	349.2	327.9	517.9	549.0	281.5	387.4	298.0	685.3
Methionine Sulfoxide	-	-	-	-	-	-	-	-	-	-
Hydroxyproline	3.1	2.7	4.4	4.6	3.1	2.5	1.9	1.8	2.6	2.8
Aspartic Acid	.6	1.3	1.2	1.1	1.3	.7	.8	.8	1.2	1.4
Threonine	5.2	3.2	7.5	8.1	10.1	6.0	4.1	6.4	7.9	10.4
Serine	7.7	8.1	10.8	9.7	11.6	8.4	9.1	9.4	9.2	11.5
Asparagine	28.3	28.0	46.2	31.6	30.3	34.4	31.8	33.9	33.1	17.4
Sarcosine	-	-	-	1.7	-	-	-	-	-	-
Proline	7.0	6.3	9.3	9.0	8.3	7.9	6.7	7.2	7.7	8.3
Glutamic Acid	6.9	10.8	9.1	8.8	12.0	6.9	4.8	7.7	9.3	9.1
Citrulline	6.2	3.5	6.4	7.4	7.8	5.1	3.4	5.7	4.7	5.8
Glycine	33.0	37.7	46.0	38.7	36.9	41.5	31.9	42.6	47.6	38.6

TABLE 2 (Cont'd)

Alanine	25.4	18.6	27.5	22.0	27.8	31.1	14.8	18.0	24.0	26.8
α -Aminoadipic Acid	.2	.2	.2	.2	-	.1	-	-	.2	.3
α -Amino-n-butyric Acid	.5	.6	.8	.1	.8	.5	.6	.1	.4	.5
Valine	22.7	15.6	22.6	35.1	31.7	20.8	19.4	22.4	28.3	30.7
Half-Cystine	-	-	-	-	-	-	-	-	-	-
Cystathionine	.8	.8	.9	.9	trace	.8	.7	.8	.8	.7
Methionine	3.6	2.8	3.6	3.7	5.2	3.6	2.7	3.1	3.4	3.8
Isoleucine	10.7	7.6	10.2	14.2	16.7	11.6	7.9	8.7	11.0	14.4
Leucine	13.1	12.9	20.0	22.6	21.8	13.1	16.7	20.0	16.3	20.0
Tyrosine	4.1	2.6	5.1	6.7	8.6	4.1	4.1	5.0	6.7	8.4
Phenylalanine	4.7	3.8	6.1	7.3	7.9	5.0	4.7	6.2	6.3	7.7
β -Alanine	.2	.3	.2	.2	-	.3	.2	.2	.3	.4
β -Aminoisobutyric Acid	-	-	-	-	-	-	-	-	-	-
Hydroxylysine	-	trace	trace	-	trace	-	-	-	-	trace
Allohydroxylysine	.1	-	-	-	-	-	-	-	.3	-
α -Aminobutyric Acid	-	-	-	-	-	-	-	-	-	-
Ornithine	7.5	5.4	8.5	9.4	10.1	8.2	6.6	7.9	8.8	11.4
Ethanoalanine	-	-	-	-	-	-	-	-	-	-
Ammonia	29.2	48.8	26.2	29.6	38.2	31.5	31.2	22.7	51.9	43.1

TABLE 2 (Cont'd)

Lysine	8.5	4.9	6.8	6.2	8.5	9.9	3.9	4.6	5.3	8.8
1-Methyl histidine	1.1	1.4	1.9	1.9	1.7	1.5	1.5	1.5	1.3	1.8
Histidine	3.9	4.2	6.1	5.4	4.3	4.4	5.2	4.9	4.6	4.8
3-Methyl histidine	.3	.4	.6	.2	.2	.3	.2	.3	.2	trace
Anserine	-	-	-	-	7.1	-	-	-	-	6.9
Tryptophan	-	-	-	-	-	-	-	-	-	-
Creatinine	61.1	76.6	64.8	61.2	56.8	66.9	52.0	58.6	58.6	60.9
Arginine	16.8	15.8	16.8	14.7	14.2	15.2	14.8	11.2	12.0	14.3

1/ None detectable

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Entomology Research Division
P.O. Box 986
Mission, Texas 78572

April 11, 1972

Subject: Screwworm Eradication Program Information

To: R. E. Ginrich, Research Entomologist
Kerrville, Texas

I have all the dates and figures you asked for last week on the telephone.

The first use of a liquid medium for mass rearing of screwworm flies was late 1964. The cost of materials for the present liquid diet is \$140 per million flies, nutria is also \$140/million flies, lung was \$200/million flies, and horsemeat was \$180/million flies. All these are estimates, but the horsemeat figure is more guess than the others. You have Jack's permission to cite him as the source of the above information.

In a paper by C. L. Smith (1960, JEE 53: 1110), the number of pupae produced at Bithlo was 285,615,587 and at Sebring 3,516,780,000 (total 3,802,395,587). I asked Dr. Meadows for a similar figure for Texas, and he had W. H. Sudlow compile them. From June 25, 1962, (when they started compiling the figures) through March 31, 1972, there were 71,282,100,000 flies produced at the Mission plant.

Maxwell M. Crystal
Research Entomologist
In Charge

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NATIONAL PROGRAM STAFF
Beltsville, Maryland 20705

RC Bushland
9 MAY 1973
B

April 30, 1973

Subject: Screwworm Nutrition

To: P. D. DeLay, Livestock & Veterinary Sciences
F. R. Senti, Marketing, Nutrition, & Engineering Sciences

As you know the screwworm suppression program has been in difficulty during the last two years. Last August Dr. R. C. Bushland was transferred back to Mission, Texas, to take charge of the research effort which underpins the APHIS program.

A number of corrective measures have been implemented, yet the released flies are very ineffective in competing with wild flies for mates. Studies in Mexico during January, February, and March indicated that an overflooding ratio of 300:1 had been attained, yet 50% of the egg masses were fertile. The major deficiency is the release strain itself, for it has been domesticated over the years. If all goes well this strain will be replaced by another with a broad genetic base in June.

Nevertheless there is a further problem. Artificially reared screwworms have never closely resembled wound-reared screwworms. The wound-reared fly has a metallic blue color and a broad thorax. The latter characteristic indicates that the wound-reared fly has powerful flight musculature. On the other hand, the artificially reared fly is dull, has a more narrow thorax, and does not appear to be robust. Perhaps changes in diet or rearing procedures would contribute significantly to the production of a more competitive fly.

About two months ago at an ARS-APHIS meeting on the screwworm Drs. Bushland and Knipling recommended that further nutritional studies should be made on the screwworm. Accordingly, I requested that Dr. Ray Moore, an expert on boll weevil nutrition, and Dr. Richard Gingrich (who has conducted previous studies on screwworm nutrition) review the diets and procedures used for screwworm rearing and identify further questions that should be examined. Enclosed is a copy of Dr. Moore's report of April 6. You will note that Dr. Moore would like to have comprehensive information on the chemical composition of beef blood plasma, red cells, and muscle. Specifically he would like to know (1) total protein, (2) amino acid content, (3) mineral content, (4) vitamin content, (5) cholesterol content, and (6) other components.

I should be most grateful if you would ask the proper experts to send the above information to Dr. Moore.

Dr. Moore would also appreciate quantitative analyses for total protein and for individual amino acids of wound-reared and artificially reared screwworms. Please indicate which ARS laboratories are set up to make these analyses.

I recommend that we give this matter a very high priority.

Waldemar Klassen

Waldemar Klassen
Staff Scientist
Pest Management

Enclosure

cc:

H. O. Graumann, w/encl.

E. F. Knipling, "

R. F. Moore

R. C. Bushland ✓

R. Gingrich

MAY 1973
B5 1

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHEASTERN REGION
Eastern Regional Research Center
600 East Mermaid Lane
Philadelphia, Pennsylvania 19118

May 9, 1973

Subject: Information on Composition of Beef Blood Plasma, Muscle, etc.

To: F. R. Senti, Acting Assistant Administrator
USDA, ARS
National Program Staff
Washington, D. C. 20250

7/28

This is in response to your memorandum of May 7, 1973 requesting information on the above subject.

Our Meat, Hides and Leather Laboratory does not know of any single reference text containing the compositional information you have requested on beef blood plasma, red cells and muscle, but have assembled data from several sources. Literature values in some cases appear to represent analyses of only a few samples and more data probably could be found following a more exhaustive search. We have our own data on protein, mineral, and amino acid content of beef muscle which will be included in a proposed publication, "Composition and PER Values of Partially Defatted Chopped Beef and Partially Defatted Beef Fatty Tissue", Muriel L. Happich, et al. This information on beef muscle (semitendinosus) composition is as follows:

a. Proximate composition: moisture, 72.6%; fat, 4.6%; protein, 21.8%.

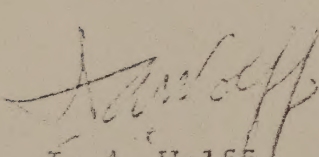
b. Amino acid analysis (as g. of amino acid residue per 100 g amino acid residues, 81.9% N accounted for) histidine, 3.6; isoleucine, 5.0; leucine, 8.3; lysine, 8.8; methionine, 2.6; 1/2 cystine, 1.3; phenylalanine, 4.9; tyrosine, 3.9; threonine, 4.4; tryptophan, 1.3; and valine, 5.5.

Copies have been made of material appearing in the literature and are attached herewith. The subject matter and the pertinent references are listed below:

1. Composition of bovine blood:

By-Products of the Meat Packing Industry. Prepared and Edited by the Committee on Textbooks of the American Meat Institute. 1958, pp 400-402.

2. Proteins in cattle blood:
Biochemists' Handbook, Edited by Cyril Long, D. Van Nostrand Co., Inc., N. Y. 1961, pp 839-842.
3. Amino acid content of blood and muscle proteins:
Amino Acid Handbook, R. J. Block, K. W. Weiss, H. J. Almquist, D. B. Carroll, W. B. Gordon and S. Sapestein. C. H. Thomas, Springfield, Illinois, 1956. pp. 252-257, 341.
4. Protein and minerals in beef muscles:
Factors Affecting the Water Retention of Beef. 1. Variations in Composition and Properties Among Eight Muscles. C. E. Swift and M. D. Berman, Food Tech. 13, 365-370 (1959).
5. Vitamin content of beef:
The Science of Meat and Meat Products. 2nd Ed., Edited by J. F. Price and B. S. Schweigert. W. H. Freeman and Company, 1971 pp 306-308.
6. Cholesterol content of beef muscle:
Composition of Foods. Agriculture Handbook No. 8, B. K. Watts and A. L. Merrill, ARS, USDA, 1963, p 146.
7. Cholesterol content of beef muscle:
Free and Esterified Cholesterol Content of Animal Muscles and Meat Products, C. Tu, W. D. Powrie, and O. Fennema. J. Food Sci., 32, 30-34 (1967).


I. A. Wolff
Area Director

7 Enclosures

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 11, 1973

Subject: Screwworm Nutrition

To: R. F. Moore, Jr.

Through: H. M. Taft, Research Leader
SE Cotton Insects Investigations

I am sorry for being so slow in replying to your letter of April 11.

I am in full agreement with the summary you wrote to Dr. Klassen on April 6 and concur in the plan you proposed to me on April 11. However I delayed my reply until I could assure you of a definite time that we could ship you insects reared on sheep. I have now completed the arrangements and the sheep will be infested tomorrow. Grown larvae will be collected and preserved on May 18 and adult flies will be ready to ship about May 29. Simultaneously we will rear screwworms from the very same culture in the laboratory. I will be out of town when the insects are ready, so Dr. Crystal will preserve and ship them according to your directions.

We have one question about the "closed vial." It seems that bacterial decomposition might be a factor if the flies were merely frozen and then put into a vial at room temperature for a couple of days. We think that it would be better to put the flies in a vial alive, kill them by freezing, and then send the vial to you packed in dry ice. The insects preserved in chloroform:methanol would be sent in the same package unless you think freezing enroute would harm them.

R. C. Bushland
Research Leader

cc Dr. Klassen

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
~~ENTOMOLOGICAL RESEARCH DIVISION~~
PEE DEE EXPERIMENT STATION
P. O. BOX 271
FLORENCE, SOUTH CAROLINA 29501

6 APR 1973
B

April 11, 1973

Subject: Screwworm Nutrition

To: R. C. Bushland, Mission, Texas

Through: H. M. Taft, Research Leader
SE Cotton Insects Investigations

I enjoyed visiting the Screwworm Research Laboratory and appreciated the courtesies which were extended.

A copy of the report to Dr. Klassen is enclosed.

I have read a review article about insect pigments (Ann. Rev. Ent. 17: 403, 1972) and believe that the nature of the color in the adult fly should be determined. If the color is due to pigments, then the amino acids tryptophan, tyrosine, cystine and methionine may be involved. Comparison of pigments from wound and lab-reared might then suggest nutrients which should be added to the diet.

We did not discuss the lipids of the screwworm to any great length and I don't believe that they are a significant factor in the color and size of the adult fly. However, I would suggest that the lipids be examined to insure that no significant points be overlooked.

I believe it would be possible to determine if pigments are a part of the color of the screwworm here at the SE Cotton Insects Investigations. The lab is also equipped to do lipid analysis.

If you agree that these things should be done then I would appreciate receiving some wound and lab-reared larvae and flies. The larvae and adults for lipid analysis should be weighed and then put in a volume of chloroform:methanol (2:1) equal to 3 x their weight. The ones for studies of the cuticular pigments could be killed by freezing and then shipped in a closed vial. A minimum of 10 individuals in each group would be adequate for one test. I would suggest the following groups:

1. Lab-reared
 - a. mature larvae in chloroform:methanol (2:1)
 - b. adult flies " " "
 - c. adult flies frozen, in a closed vial
2. Wound-reared
 - a. mature larvae in chloroform:methanol (2:1)
 - b. adult flies " " "
 - c. adult flies frozen, in a closed vial

Your comments will be appreciated.

R. F. Moore, Jr.
R. F. Moore, Jr.
Research Entomologist

cc: W. Klassen

XXXXXXXXXXXXXXXXXXXXXXXXXXXX

April 6, 1973

Subject: Screwworm Nutrition

**To: Dr. Waldemar Klassen
USDA- National Program Staff
Beltsville, Maryland 20705**

**Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Investigations**

I arrived in McAllen, Texas on March 29, 1973 and visited the Mission Rearing Facility the following day. In the morning, Mr. Frank Dudley, Bio-technician, Fly Production gave me a tour of the plant and an excellent explanation of the rearing process and of the problems in this program. In the afternoon I visited with Dr. M. M. Crystal, Research Entomologist, and Mr. G. W. Eddy, Research Entomologist, both of the Screwworm Research Laboratory and Dr. H. C. Hoffman, Research Entomologist, Methods Development. The weekend was spent in studying materials supplied by ARS and APHIS.

On Monday, Apr. 2, 1973, Dr. R. E. Gingrich from Kerrville, Texas and I discussed screwworm nutrition with Dr. R. C. Bushland. Later in the day we talked with Mr. A. J. Graham, Staff Entomologist.

These discussions produced the following objectives and proposed approach to achieve the objectives:

The primary objective is to develop a series of nutritional experiments that would have the potential of producing, from an artificial diet, a fly that resembles the wild fly more closely than those now reared on artificial media. Specifically, a fly which has the "blue" color and "broad" thorax of the wild fly. It was suggested that the "blue" color may have a value in mate selection in the wild and that the "broad" thorax might indicate a strong capacity for flight and hence a fly that is able to compete effectively for mates. It is also possible that these visible defects indicate that there are other invisible biochemical defects which reduce the competitiveness of the lab-reared fly.

A secondary objective is to evaluate and suggest sources of protein that would be more economical than that currently in use but still produce a male fly that is competitive with the wild fly.

Objective 1

Screwworm flies produced on artificial diets have been found to have a more narrow thorax and to be more green in color than those reared in the wound of a living animal. Diets containing meat should be most comparable to the natural diet. Therefore, it is suggested that efforts be made to obtain a wild type of adult fly by modification of a meat diet.

There are 4 areas where modifications could be made that might result in the desired type of fly. These, in suggested order of importance, are: (1) nutrition, (2) minerals, (3) vitamins, and (4) microbial inhibitors. Selection of the modifications to be made to the meat diet in nutrition requires the following information: A. Analysis of beef blood plasma, red cells, and muscle for total protein, amino acid content, mineral content, selected vitamins, cholesterol, and other components that might be suggested by a more complete search of the literature; B. Analyses of larvae and adults of wild screwworm flies for total protein and amino acids; C. Analysis of larvae and adults of lab-reared insects for total protein and amino acids; and D. Separation of proteins by electrophoresis from wild and lab flies might give useful information if differences are found.

It is requested that you determine if there is someone on the National Program Staff who will be able to locate the information required in A. Also, could you determine if the analyses required in B and C can be done at a laboratory of the Department of Agriculture? The information would be used to adjust the balance of essential amino acids and insure an adequate supply of those that might be growth limiting such as lysine and methionine.

Modifications in the minerals will be based on information in the literature; the objective is to insure that adequate trace elements are included in the media. Dilution and loss of some vitamins is almost certain, so it is suggested that a complete mixture be added. Microbial inhibitors would have the objective of preventing decay and bacterial contamination and the associated change in nutritional value of the diet. Their selection would be aided by results from the literature.

Design and testing of a device to produce a slow, constant flow of blood plasma over the larvae might be desirable if the above changes are not successful. Such a device would have the effect of constantly removing waste products and approximating the natural environment more closely.

Objective 2

The information on amino acids obtained for objective No. 1 and the published amino acid mixture of Dr. Gingrich will be useful in attaining this objective.

It is suggested that various sources of protein such as soybean, Child Food Supplement #2 (corn-soybean-milk), Wheast[®], and others be analyzed for total protein and amino acid content. These proteins might then be enriched with essential amino acids that are limiting and then be used as a more economical protein than is now available.

Miscellaneous

The liquid diet now uses linters cotton as a substrate for the screwworm larvae. The diet is changed by vacuuming off about one-half of the spent liquid and then fresh diet is added. This results in lost nutrients in the portion removed and the fresh diet is diluted with the wastes of the diet that was left. Recently, several companies have developed a process for putting soybean protein into a form that has the texture of meat. Use of this technique might make it possible to put the liquid diet into a semi-solid form and permit the larvae to move from the spent to fresh diet as now occurs in the diet based on meat.

It might be worthwhile to determine if the color of the adult screwworm is the result of pigments in the cuticle or the diffraction grating method of color formation. Electron microscope studies might determine this and could provide a clue to the difference in color between wild and lab-reared flies.

Action to be Taken

It is recognized that these objectives cannot be reached in time to be of value to the releases of flies scheduled for the 1973 season. More effective results are expected if required nutritional data are obtained and the literature reviewed and then the information used to design the diets to be tested. The goal is to receive this information by September 1973.

I would be willing to plan my research program at Florence, S. C. so that it would be possible for me to return to the Screwworm Research Lab at Mission, Texas during the fall of 1973 for a period of approximately one month to assist in the initiation of these experiments.

This has been discussed with Mr. A. J. Graham, Staff Entomologist, and Mr. Ed Corrigan, Entomologist in Charge of Methods Development. They have concurred in this approach and feel that it could be possible for portions of this work to be done in the Mass Rearing Plant. Dr. R. E. Gingrich has indicated that he will be available to assist.

Your comments and reactions to this proposal, either by letter or telephone will be appreciated.

R. F. Moore, Jr.
Research Entomologist

cc: H C Cox, R. C. Bushland, W. M. Bruce, A. J. Graham, Ed Corrigan,
J. Rex Johnston, E. A. Taylor, R. E. Gingrich

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 11, 1973

Subject: Screwworm Nutrition

To: R. F. Moore, Jr.

Through: H. M. Taft, Research Leader
SE Cotton Insects Investigations

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R. C. Bushland
Research Leader

cc Dr. Klassen

REFERENCE SLIP

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REMARKS

FROM

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UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
~~ENTOMOLOGICAL RESEARCH DIVISION~~
PEE DEE EXPERIMENT STATION
P. O. BOX 271
FLORENCE, SOUTH CAROLINA 29501

46 APR 1973
B

April 11, 1973

Subject: Screwworm Nutrition

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Through: H. M. Taft, Research Leader
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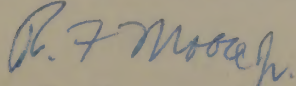
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R. F. Moore, Jr.
Research Entomologist

cc: W. Klassen

XXXXXXXXXXXXXXXXXXXXXXXXXXXX

April 6, 1973

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USDA- National Program Staff
Beltsville, Maryland 20705

Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Investigations

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A secondary objective is to evaluate and suggest sources of protein that would be more economical than that currently in use but still produce a male fly that is competitive with the wild fly.

Objective 1

Screwworm flies produced on artificial diets have been found to have a more narrow thorax and to be more green in color than those reared in the wound of a living animal. Diets containing meat should be most comparable to the natural diet. Therefore, it is suggested that efforts be made to obtain a wild type of adult fly by modification of a meat diet.

There are 4 areas where modifications could be made that might result in the desired type of fly. These, in suggested order of importance, are: (1) nutrition, (2) minerals, (3) vitamins, and (4) microbial inhibitors. Selection of the modifications to be made to the meat diet in nutrition requires the following information: A. Analysis of beef blood plasma, red cells, and muscle for total protein, amino acid content, mineral content, selected vitamins, cholesterol, and other components that might be suggested by a more complete search of the literature; B. Analyses of larvae and adults of wild screwworm flies for total protein and amino acids; C. Analysis of larvae and adults of lab-reared insects for total protein and amino acids; and D. Separation of proteins by electrophoresis from wild and lab flies might give useful information if differences are found.

It is requested that you determine if there is someone on the National Program Staff who will be able to locate the information required in A. Also, could you determine if the analyses required in B and C can be done at a laboratory of the Department of Agriculture? The information would be used to adjust the balance of essential amino acids and insure an adequate supply of those that might be growth limiting such as lysine and methionine.

Modifications in the minerals will be based on information in the literature; the objective is to insure that adequate trace elements are included in the media. Dilution and loss of some vitamins is almost certain, so it is suggested that a complete mixture be added. Microbial inhibitors would have the objective of preventing decay and bacterial contamination and the associated change in nutritional value of the diet. Their selection would be aided by results from the literature.

Design and testing of a device to produce a slow, constant flow of blood plasma over the larvae might be desirable if the above changes are not successful. Such a device would have the effect of constantly removing waste products and approximating the natural environment more closely.

Objective 2

The information on amino acids obtained for objective No. 1 and the published amino acid mixture of Dr. Gingrich will be useful in attaining this objective.

Objective 1

Scarcely flies produced on artificial diets have been found to have a more narrow thorax and to be more green in color than those reared in the medium of a living animal. Diets containing meat should be most comparable to the natural diet. Therefore, it is suggested that efforts be made to obtain a wild type of adult fly by modification of a meat diet.

There are a great many modifications which could be made that might result in the desired type of fly. These, in suggested order of importance, are: (1) nutrition, (2) minerals, (3) vitamins, and (4) microbial inhibitors. Selection of the modifications to be made to the meat diet in nutrition requires the following information: A. Analysis of beef blood plasma, red cells, and muscle for total protein, amino acid content, mineral content, selected vitamins, cholesterol, and other components that might be suggested by a more complete search of the literature; B. Analysis of larvae and adults of wild scarnworm flies for total protein and amino acids; C. Analysis of larvae and adults of lab-reared insects for total protein and amino acids; and D. Separation of proteins by electrophoresis from wild and lab flies might give useful information if differences are found.

It is requested that you determine if there is someone on the National Program Staff who will be able to locate the information requested in A. Also, could you determine if the analyses requested in B and C can be done at a laboratory of the Department of Agriculture? The information would be used to adjust the balance of essential amino acids and insure an adequate supply of those that might be growth limiting such as lysine and methionine.

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Design and testing of a device to produce a slow, constant flow of blood plasma over the larvae might be desirable if the above changes are not successful. Such a device would have the effect of constantly removing waste products and approximating the natural environment more closely.

Objective 2

The information on amino acids obtained for objective No. 1 and the published amino acid mixture of Dr. Elgin will be useful in attaining this objective.

It is suggested that various sources of protein such as soybean, Child Food Supplement #2 (corn-soybean-milk), Wheat[®], and others be analyzed for total protein and amino acid content. These proteins might then be enriched with essential amino acids that are limiting and then be used as a more economical protein than is now available.

Miscellaneous

The liquid diet now uses linters cotton as a substrate for the screwworm larvae. The diet is changed by vacuuming off about one-half of the spent liquid and then fresh diet is added. This results in lost nutrients in the portion removed and the fresh diet is diluted with the wastes of the diet that was left. Recently, several companies have developed a process for putting soybean protein into a form that has the texture of meat. Use of this technique might make it possible to put the liquid diet into a semi-solid form and permit the larvae to move from the spent to fresh diet as now occurs in the diet based on meat.

It might be worthwhile to determine if the color of the adult screwworm is the result of pigments in the cuticle or the diffraction grating method of color formation. Electron microscope studies might determine this and could provide a clue to the difference in color between wild and lab-reared flies.

Action to be Taken

It is recognized that these objectives cannot be reached in time to be of value to the releases of flies scheduled for the 1973 season. More effective results are expected if required nutritional data are obtained and the literature reviewed and then the information used to design the diets to be tested. The goal is to receive this information by September 1973.

I would be willing to plan my research program at Florence, S. C. so that it would be possible for me to return to the Screwworm Research Lab at Mission, Texas during the fall of 1973 for a period of approximately one month to assist in the initiation of these experiments.

This has been discussed with Mr. A. J. Graham, Staff Entomologist, and Mr. Ed Corrigan, Entomologist in Charge of Methods Development. They have concurred in this approach and feel that it could be possible for portions of this work to be done in the Mass Rearing Plant. Dr. R. E. Gingrich has indicated that he will be available to assist.

Your comments and reactions to this proposal, either by letter or telephone will be appreciated.

R. F. Moore, Jr.
Research Entomologist

cc: H C Cox, R. C. Bushland, W. M. Bruce, A. J. Graham, Ed Corrigan,
J. Rex Johnston, E. A. Taylor, R. E. Gingrich

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Miscellaneous

The liquid diet now uses linseed cotton as a substrate for the acromioclavicular joint. The diet is changed by vacuuming off about one-half of the spent liquid and then fresh diet is added. This results in lost nutrients in the portion removed and the fresh diet is diluted with the wastes of the diet that was left. Recently, several companies have developed a process for putting soybean protein into a form that has the texture of meat. Use of this technique might make it possible to put the liquid diet into a solid-solid form and permit the larvae to move from the spent to fresh diet as now occurs in the diet based on meat.

It might be worthwhile to determine if the color of the adult acromioclavicular joint is the result of pigments in the cuticle or the diffusion grating method of color formation. Electron microscope studies might determine this and could provide a clue to the difference in color between wild and lab-reared flies.

Action to be Taken

It is recognized that these objectives cannot be reached in time to be of value to the release of flies scheduled for the 1973 season. More effective results are expected if required nutritional data are obtained and the literature reviewed and then the information used to design the diets to be tested. The goal is to receive this information by September 1973.

I would be willing to plan my research program at Florence, S. C. so that it would be possible for me to return to the Entomology Research Lab at Mission, Texas during the fall of 1973 for a period of approximately one month to assist in the initiation of these experiments.

This has been discussed with Mr. A. J. Graham, Staff Entomologist, and Mr. Ed Corrigan, Entomologist in Charge of Methods Development. They have concurred in this approach and feel that it could be possible for portions of this work to be done in the Mass Hatching Plant. Dr. R. E. Glasgow has indicated that he will be available to assist.

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J. Rex Johnston, R. A. Taylor, R. E. Glasgow

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

December 27, 1973

Subject: Dr. Ray Moore's Insect Nutrition Studies

To: Waldemar Klassen
USDA, ARS, National Program Staff
Beltsville, Maryland 20705

Dr. Moore returned to Florence, S. C., two weeks ago. I assume that by now you have received his preliminary report.

Although Dr. Moore did not solve the problem of artificially rearing flies that resemble wound-reared insects, he made a very good start. I was greatly impressed with his ability and dedication. He is continuing the work in absencia guiding our technician, Bobby Handke, by long distance telephone for another month.

Dr. Meadows of APHIS, after hearing Dr. Moore's final report said that if ARS couldn't permanently assign a nutritionist of Dr. Moore's calibre to work at Mission, that he would request such a person for his APHIS Methods Development Staff.

When the Mission laboratory has its informal program review on January 22 and 23, I hope that you can arrange for Dr. Moore to be here to discuss screwworm rearing.

R. C. Bushland
Research Leader

cc: E. A. Taylor
L. S. Henderson
Ray Moore

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

NATIONAL PROGRAM STAFF

Washington, D. C. 20250

85 MAY 1973

May 17, 1973

Subject: Screwworm Nutrition

To: R. F. Moore, Jr., Research Entomologist
ARS - USDA - Pee Dee Experiment Station
P. O. Box 271
Florence, South Carolina 29501

Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Investigations

In response to the memorandum from Dr. Waldemar Klassen to me on the above subject, I requested the Eastern Regional Research Center, Wyndmoor, Pennsylvania, to provide information on the composition of beef blood plasma, red cells, and muscle. Dr. I. A. Wolff, Area Director, ERRC, has submitted the enclosed information which they have collected from the literature. If you have questions on this compositional data, I suggest you correspond directly with Dr. Wolff.

Dr. Klassen also inquired regarding which ARS laboratories are set up to make quantitative analysis for total protein and individual amino acids in wound-reared and artificially-reared screwworms. I have discussed this subject with Dr. R. J. Dimler, Area Director, Northern Regional Research Center, Peoria, Illinois. I suggest he be contacted directly concerning the detailed proposals of analysis which may be needed.

F. R. Senti

F. R. Senti
Acting Assistant Administrator

7 Enclosures

cc:

Dr. Klassen--NPS ✓
Dr. Graumann--NPS
Dr. Wolff--ERRC
Dr. Dimler--NRRC

Mr. Glover--NCRO
Dr. Cooper--SRO
Dr. King--NERO
Dr. Taft--RL
Mr. Bruce--AD

25 MAY 1973

U.S. DEPARTMENT OF AGRICULTURE

DATE

REFERENCE SLIP

5/22/73

TO

R C Bushland

- | | |
|--|---|
| ☐ ACTION | ☒ NOTE AND RETURN |
| ☐ APPROVAL | ☐ PER PHONE CALL |
| ☐ AS REQUESTED | ☐ RECOMMENDATION |
| ☐ FOR COMMENT | ☐ REPLY FOR SIGNATURE OF |
| ☐ FOR INFORMATION | ☐ RETURNED |
| ☐ INITIALS | ☐ SEE ME |
| ☐ NOTE AND FILE | ☐ YOUR SIGNATURE |

REMARKS

Let's not lose
momentum on this
problem

FROM

W Klassen

25 MAY 1973
B

XXXXXXXXXXXXXXXXXXXXXXXXXXXX

May 22, 1973

Subject: Amino Analysis of Screwworms

To: Dr. R. J. Dimaler, Area Director
Northern Regional Research Center
Peoria, Illinois

Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Investigations

Dr. R. F. Knippling and Dr. R. C. Bushland have determined that additional research on the nutrition of the screwworm fly is desirable. Dr. R. E. Gingrich, Kerrville, Texas and I have been asked to assist in this research.

Dr. Gingrich and I visited the Screwworm Research Laboratory Apr. 2-4, 1973 and met with Dr. Bushland. It was agreed that nutritional experiments should be conducted and would begin this fall. Information to aid the design of the experiments is to be secured prior to the start of the experiments.

Comparison of total protein and amino acid content between lab-reared insects and those reared on animals is part of the needed information. Larvae and adults reared on animals would be compared with comparable stages reared on artificial media. It is expected that this comparison should indicate gross deficiencies of amino acids that may be present in the lab-reared insects.

Dr. F. E. Senti, in a letter dated May 17, 1973, has suggested that you be contacted concerning an amino acid analysis of these insects. Is there a laboratory in the North Central Region that could perform an amino acid analysis of these insects? The insects would be supplied through the Screwworm Research Laboratory, Mission, Texas. Two larval stages and the adult should be examined. Thus, the analysis would require examination of 6 different samples and sufficient replication to insure confidence in the results.

Any assistance which you can give will be appreciated. I will be glad to try to furnish any additional information which you may require.

R. F. Moore, Jr.
Research Entomologist

cc:
Mr. Bruce
✓ Dr. Bushland
Dr. Cooper
Dr. Cox
Dr. Klanssen
Dr. Senti

25 MAY 1973
B.

XXXXXXXXXXXXXXXXXXXXXXXXXXXX

May 22, 1973

Subject: Amino Analysis of Screwworms

To: Dr. R. J. Dimler, Area Director
Northern Regional Research Center
Peoria, Illinois

Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Investigations

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R. F. Moore, Jr.
Research Entomologist

cc:

Mr. Bruce
✓ Dr. Bushland

Dr. Cooper
Dr. Cox

Dr. Klassen
Dr. Senti

25 MAY 1973

U.S. DEPARTMENT OF AGRICULTURE

DATE

5/22/73

REFERENCE SLIP

TO

R C Bushland

☐ ACTION

☒ NOTE AND RETURN

☐ APPROVAL

☐ PER PHONE CALL

☐ AS REQUESTED

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☐ FOR COMMENT

☐ REPLY FOR SIGNATURE OF

☐ FOR INFORMATION

☐ RETURNED

☐ INITIALS

☐ SEE ME

☐ NOTE AND FILE

☐ YOUR SIGNATURE

REMARKS

Let's not lose
momentum on this
problem

FROM

W Klassen

08 MAY 1973

U.S. DEPARTMENT OF AGRICULTURE

1973

REFERENCE SLIP

K. C. BAKER

NOTE CARD RETURN ☒

NOTE ☐

FOR FRONT CARD ☐

REPLY ☐

REPLY CARD ☐

REPLY CARD ☐

REPLY FOR BUREAU ☐

FOR BUREAU ☐

REPLY ☐

REPLY ☐

SEE ME ☐

THANKS ☐

YOUR SIGNATURE ☐

YOUR NAME ☐

John A. Baker

1973

1973

File "Screwworm"

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NATIONAL PROGRAM STAFF
Washington, D. C. 20250

25 MAY 1973

May 17, 1973

Subject: Screwworm Nutrition

To: R. F. Moore, Jr., Research Entomologist
ARS - USDA - Pee Dee Experiment Station
P. O. Box 271
Florence, South Carolina 29501

Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Investigations

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F. R. Senti

F. R. Senti
Acting Assistant Administrator

7 Enclosures

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Dr. Klassen--NPS ✓
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Dr. Cooper--SRO
Dr. King--NERO
Dr. Taft--RL
Mr. Bruce--AD

25 MAY 1973
25 1

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHEASTERN REGION
Eastern Regional Research Center
600 East Mermaid Lane
Philadelphia, Pennsylvania 19118

May 9, 1973

Subject: Information on Composition of Beef Blood Plasma, Muscle, etc.

To: F. R. Senti, Acting Assistant Administrator
USDA, ARS
National Program Staff
Washington, D. C. 20250

JRS

This is in response to your memorandum of May 7, 1973 requesting information on the above subject.

Our Meat, Hides and Leather Laboratory does not know of any single reference text containing the compositional information you have requested on beef blood plasma, red cells and muscle, but have assembled data from several sources. Literature values in some cases appear to represent analyses of only a few samples and more data probably could be found following a more exhaustive search. We have our own data on protein, mineral, and amino acid content of beef muscle which will be included in a proposed publication, "Composition and PER Values of Partially Defatted Chopped Beef and Partially Defatted Beef Fatty Tissue", Muriel L. Happich, et al. This information on beef muscle (semitendinosus) composition is as follows:

a. Proximate composition: moisture, 72.6%; fat, 4.6%; protein, 21.8%.

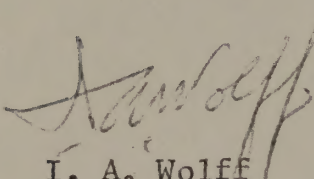
b. Amino acid analysis (as g. of amino acid residue per 100 g amino acid residues, 81.9% N accounted for) histidine, 3.6; isoleucine, 5.0; leucine, 8.3; lysine, 8.8; methionine, 2.6; 1/2 cystine, 1.3; phenylalanine, 4.9; tyrosine, 3.9; threonine, 4.4; tryptophan, 1.3; and valine, 5.5.

Copies have been made of material appearing in the literature and are attached herewith. The subject matter and the pertinent references are listed below:

1. Composition of bovine blood:

By-Products of the Meat Packing Industry. Prepared and Edited by the Committee on Textbooks of the American Meat Institute. 1958, pp 400-402.

2. Proteins in cattle blood:
Biochemists' Handbook, Edited by Cyril Long, D. Van Nostrand Co., Inc., N. Y. 1961, pp 839-842.
3. Amino acid content of blood and muscle proteins:
Amino Acid Handbook, R. J. Block, K. W. Weiss, H. J. Almquist, D. B. Carroll, W. B. Gordon and S. Sapestein. C. H. Thomas, Springfield, Illinois, 1956. pp. 252-257, 341.
4. Protein and minerals in beef muscles:
Factors Affecting the Water Retention of Beef. 1. Variations in Composition and Properties Among Eight Muscles. C. E. Swift and M. D. Berman, Food Tech. 13, 365-370 (1959).
5. Vitamin content of beef:
The Science of Meat and Meat Products. 2nd Ed., Edited by J. F. Price and B. S. Schweigert. W. H. Freeman and Company, 1971 pp 306-308.
6. Cholesterol content of beef muscle:
Composition of Foods. Agriculture Handbook No. 8, B. K. Watts and A. L. Merrill, ARS, USDA, 1963, p 146.
7. Cholesterol content of beef muscle:
Free and Esterified Cholesterol Content of Animal Muscles and Meat Products, C. Tu, W. D. Powrie, and O. Fennema. J. Food Sci., 32, 30-34 (1967).


I. A. Wolff
Area Director

7 Enclosures

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
~~ENTOMOLOGICAL RESEARCH DIVISION~~
PEE DEE EXPERIMENT STATION
P. O. BOX 271
FLORENCE, SOUTH CAROLINA 29501

27 AUG 1973

August 22, 1973

Subject: Nutritional Experiments With the Screwworm Fly

To: Dr. R. C. Bushland, Research Leader
Screwworm Research Laboratory
P. O. Box 986, Mission, Texas 78572

Through: H. M. Taft, Research Leader
SE Cotton Insects Investigations

Summer activities are almost over and I feel we should begin to plan and make a tentative schedule for the subject experiments.

In the next few weeks I hope to send you and Dr. Gingrich a summary of my thoughts about some factors which might play a role in the color and robust appearance of wild adult flies. The ideas from the three of us, the amino acid data and suggestions from the literature should give us a good selection of variables for testing. We need to establish some specific idea of the nutrients and chemicals that will be tested so they can be ordered and be available when we are ready to begin the experiments.

Have you received any of the amino acid data from Mr. James Cavin? I have some preliminary data from the flies which you sent. There is a lipid in the extract from sheep-reared flies that is absent from the media-reared flies. I don't know what it is or its significance. I will attempt to identify it and also determine fatty acids and triglycerides in the various larvae and adults. Acid and alkaline hydrolysis destroys the color in the cuticle of the flies which suggests that pigments produce the color. I plan to make some more tests on this. If the color is due to pigments then the difference in color between animal- and medium-reared flies may be due to differences in the availability of certain amino acids. I plan to have more definite data in the immediate future.

I am thinking of a date around Oct. 15, 1973 for the start of these studies. A week earlier or later would be equally acceptable. This should give us time to complete the studies before the end of the year. The first day would be well spent if Dr. Gingrich, yourself, and any others that would be participating in the experiments could meet and discuss what is to be done, where, the technical assistance that will be necessary, and other pertinent matters. Thus, the best time for me to come will depend somewhat on Dr. Gingrich's schedule

2.

and that of the personnel at Mission. Perhaps Dr. Gingrich can let you know his schedule and you can determine the most suitable time for the personnel at Mission and let me know the time you have selected.

I am assuming that funds have been provided your laboratory for my travel and per diem. Please let me know how you wish this to be handled. The lodging at Sheraton-Fairway in March was fine and will be suitable again if you feel this is the best arrangement. I could drive my personal car to Mission if it would be useful. Then it would not be necessary for you to furnish me transportation between the motel and the laboratory at Mission. I could also bring in the car some miscellaneous items that would not be convenient to bring on a plane. These items would be selected for their possible usefulness in the experiments. They may or may not be needed but in any case would not be taken on a plane. I'd appreciate knowing your feeling about the transportation. Per diem and mileage would probably equal air fare so the chief advantage would be those things that could be brought in the car and transportation during the time I am in Texas.

Your comments on the above will be appreciated.

Ray Moore

R. F. Moore, Jr.
Research Entomologist

cc:

W. Klassen
W. M. Bruce
H C Cox
R. E. Gingrich

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

SOUTHERN REGION

U. S. Livestock Insects Laboratory

P. O. Box 232

Kerrville, Texas 78028

28 AUG 1973

August 28, 1973

Subject: Meeting on Nutrition of Screwworms

To: R. C. Bushland, Research Leader
Screwworm Research Laboratory, ARS, USDA
Mission, Texas

Through: R. O. Drummond, Research Leader, *RCB* U. S. Livestock Insects Laboratory

Dr. R. F. Moore has requested that I arrange with you a time around the middle of October for discussing plans for the forthcoming experiments on screwworm nutrition. Any time the week of the 15th would be agreeable with me. I will be gone the first week of October, but the next month is open.

I hope this time is acceptable to you. Best regards.

R. E. Gingrich
8/6

R. E. Gingrich
Research Entomologist

8/31

*Answered by copy of letter 8/28
to Moore*

RCB

Screwworm Research Laboratory
P.O. Box 986
Mission, Texas 78572

August 28, 1973

Subject: Nutritional Experiments with the Screwworm Fly

To: R. F. Moore, Jr., Research Entomologist
SE Cotton Insects Investigations

Through: H. M. Taft, Research Leader
SE Cotton Insects Investigations

Thank you for your letter of August 22 proposing that you begin your month's temporary duty at this laboratory approximately October 15. I will be away from the laboratory October 15 through October 23, so suggest that you come either the week before my departure or any time you wish after October 23.

The most important factor is that your trip be coordinated with Dr. Gingrich so that he can work with you at the beginning of your experiments.

The texture soy protein and letter of transmittal came last week. I delivered both to Mr. A. J. Graham of APHIS. He will conduct a preliminary trial. Earlier he tested some soy flour with encouraging results as far as larval growth is concerned.

These soy products may be too expensive. The material you sent was priced at 30 to 60 cents a pound. If 60 cents is the more realistic figure, then the rehydrated product simulating meat after taking up 3 parts of water would cost 15 cents per pound. The screwworm program bought frozen nutria for 11 cents a pound last year. It will probably be higher this year, and the supply is not adequate so there is good reason to consider soy protein.

We did not get any amino acid data from Mr. Cavin, so you may want to check with him again before you come here.

As far as I am concerned, it will be fine for you to come in your car and collect mileage. I am referring your letter and this reply to the Area Director, Mr. E. A. Taylor, who controls our finances. Unless he notifies me to the contrary, I'll assume that we can pay your travel for approximately one month here this fall.

R. C. Bushland
Research Leader

CC: E. A. Taylor

Screwworm Research Laboratory
P.O. Box 986
Mission, Texas 78572

August 28, 1973

Subject: Nutritional Experiments with the Screwworm Fly

To: R. F. Moore, Jr., Research Entomologist
SE Cotton Insect Investigations

Through: H. M. Telf, Research Leader
SE Cotton Insect Investigations

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We did not get any amino acid data from Mr. Gavin, so you may want to check with him again before you come here.

As far as I am concerned, it will be fine for you to come in your car and collect mileage. I am referring your letter and this reply to the Area Director, Mr. E. A. Taylor, who controls our finances. Unless he notifies me to the contrary, I'll assume that we can pay your travel for approximately one month here this fall.

R. C. Bushland
Research Leader

CC: E. A. Taylor

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
~~ENTOMOLOGICAL RESEARCH DIVISION~~
PEE DEE EXPERIMENT STATION
P. O. BOX 271
FLORENCE, SOUTH CAROLINA 29501

17 SEP 1973

B.

September 10, 1973

Subject: Nutritional Experiments on Screwworm Flies

To: R. C. Bushland, Laboratory for Screwworm Research
Mission, Texas

Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Investigations

As we have agreed, the objectives of the subject experiments are to obtain from media, adult flies that have the blue color and the robust appearance of flies reared in an animal and hopefully this will result in a "normal" fully competitive fly.

I propose that the primary approach to these objectives be based on the premise that the flies reared on media get insufficient amino acids from their diets. The premise is suggested by the following. First, I recall that you did not remember any media-reared flies looking like animal-reared flies, even on the very best media and under the best conditions. Conditions in a living tissue are very different from those in dead tissue and an unknown factor related to this may be the basis for the differences in the flies. However, in all the diets which I have seen, plasma or blood is only 5-30% of the liquid in the diet. My impression is that in the living animal, the larvae are in the flesh and bathed in lymph or plasma. This seems significant because published values for the amino acid composition of beef muscle and blood plasma show that the plasma contains higher percentages of cystine, leucine, phenylalanine, tryptophan, tyrosine and valine. The plasma contains approximately 7% protein which is diluted by at least one-third in most diets. Dick Gingrich showed that cystine, leucine, phenylalanine, tryptophan and valine are among the amino acids required by the screwworm. Thus, there is a possibility that dilution of the plasma results in the media being deficient in the amino acids mentioned above.

It is my impression that the robust appearance of the fly is related to the wideness of the thorax. The only thing which I have found that is related to this is the finding of S. D. Beck that the head capsule was narrow in the larvae of the European corn borer when reared on diets that were deficient in protein or carbohydrate. This is suggestive of a role for amino acids in the robust appearance of the screwworm.

Color in insects may be due to the physical characteristics of the exoskeleton or to pigments. Some simple tests that I have done on the screwworm suggest that both of these contribute to the color of the fly. The

2.

bluish-green color of the adult changes to a golden brown in 10% sodium hydroxide and to a greenish-yellow in 6 N HCl. Changing from acid to alkaline will reverse the colors and this can be repeated several times on the same piece of exoskeleton. This is suggestive of pigments. When the golden brown cuticle in alkaline solution is rotated to a particular angle, a blue color can be seen and this suggests a physical factor. A recent review of pigments (Fuzeau-LBraesch, 1972, Ann. Rev. Ent. 17:403) indicates that tryptophan, cystine, methionine and tyrosine are involved in pigment formation. Another article implicates phenylalanine. It is possible that all of these except methionine are deficient in the media. Bile pigments are also involved in color in some insects and it is possible that such pigments in whole dried blood may be affecting the color of the flies.

Thus, there is evidence for the possible deficiency of certain amino acids in the media and there are indications that the difference in color and appearance of the flies could be caused by such a deficiency.

An experiment using undiluted plasma as the liquid in a meat diet should be done if it has not already been tried. A parallel experiment to develop an artificial plasma is suggested. This should be based on 6-7% egg albumin plus any amino acids necessary to adjust it to a composition similar to that of plasma. The plasma would be made isotonic with Wesson salt mixture plus CoCl_2 , Zn-acetate and Na molybdate. Cholesterol and vitamins would also be added. This artificial plasma could be substituted for the blood plasma in a meat media. If these diets are successful in producing a "normal" fly, then the artificial plasma could serve as a basis for determining which amino acids were responsible for the color differences and the robust appearance and could possibly serve as a means of evaluating proteins for use in other diets.

Do you think it would be wise to devise a color measurement test so that there would be some objective means of determining how closely the color of the media flies resembles that of animal reared? Would measurement of the length and width of the thorax or the resulting ratio give a valid measure of the robustness of the flies?

I hope that you and Dick Gingrich will be critical of these proposals and point out deficiencies in information or errors in reasoning.

The amino acids, vitamins, etc. will be ordered here and I will bring them with me when I come to Texas.

R. F. Moore, Jr.

R. F. Moore, Jr.
Research Entomologist

cc:
Dick Gingrich
W. Klassen

17 SEP 1973

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
~~ENTOMOLOGICAL RESEARCH DIVISION~~
PEE DEE EXPERIMENT STATION
P. O. BOX 271
FLORENCE, SOUTH CAROLINA 29501

B

September 10, 1973

Subject: Nutritional Experiments on Screwworm Flies

To: R. C. Bushland, Laboratory for Screwworm Research
Mission, Texas

Through: H. M. Taft, Research Leader
Southeastern Cotton Insects Investigations

HT

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R. F. Moore, Jr.

R. F. Moore, Jr.
Research Entomologist

cc:
Dick Gingrich
W. Klassen

Dr. C. M. Smith

UNITED STATES DEPARTMENT OF AGRICULTURE 61 OCT 1973

AGRICULTURAL RESEARCH SERVICE

Southern Region

Georgia-South Carolina Area

Southeastern Cotton Insects Investigations

P. O. Box 271, Florence, S. C. 29501

September 25, 1973

Subject: Nutritional Experiments with the Screwworm Fly

To: R. C. Bushland, Research Leader
Lab. for Screwworm Research, Mission, Texas

Through: H. M. Taft, Research Leader
SE Cotton Insects Investigations

On Sept. 12 I had an opportunity to talk with Alfred Baumhover about his experiences in the screwworm program and the proposed series of nutritional experiments. During the week of Sept. 17 I visited the Dept. of Entomology at North Carolina State University, Raleigh, N. C. and discussed the subject experiments with Dr. Ernest Hodgson, Dr. Robert Yamamoto, and Dr. G. C. Rock. Later, I talked with Dr. James Vaughn and Dr. Ron Goodwin in Insect Pathology and Dr. Bill Robbins, Dr. Jim Svoboda, and Dr. John Kaplanis in Insect Physiology at Beltsville, Maryland.

These discussions were generally useful and some additional insights and information were gained.

Listed below in order of suggested priority are some specific experimental diets. The number to be tested will depend on the availability of technical assistance, the number of diets that can be tested at one time and the number of insects and replicates. Some selection will probably be necessary.

The suggested diets are:

1. Horse meat with plasma diluted with water.
2. Horse meat with undiluted plasma.
3. Horse meat with undiluted plasma flowing through the meat.
(I will attempt to devise an apparatus for this)
4. Same as #1 but dilute plasma with a protein solution based on egg albumin and amino acids instead of water.
5. Same as #1 but dilute plasma with water made isotonic with a salt solution.
6. Same as #1 but dilute plasma with a vitamin solution.
7. Same as #1 but combine proteins, minerals and vitamins from 4, 5, and 6 in the water for dilution of the plasma.
8. Same as #2 but put a culture of Proteus chandleri in the plasma.
9. Substitute proteins, minerals and vitamins from #7 for all of the plasma.

2.

Each of the diets has a specific variable that will be tested. Comparison of media-reared flies with animal-reared flies will be necessary, but Diet #1 can also be used to determine if any of the diets produce a fly that is improved in color or the characteristic of the thorax. Diet #2 should show if dilution of some component in the plasma is responsible for the difference in media-reared and animal-reared flies. Diet #3 would provide a mechanism for removal of waste products. Selective uptake from the plasma of an unknown component that is present in very small amounts would also be a factor in this diet. Diet #s 4, 5, and 6 would test specific groups of nutrients that would be diluted by the water in Diet #1. Diet #7 would test the combination of components in #s 4, 5, and 6 and would be an approach to an "artificial plasma". Diet #9 would determine if the artificial plasma could be substituted for plasma. Diet #8 is for the purpose of determining if this micro-organism contributes to the nutrition of the fly.

The variables - weight of larvae, color of adults, and appearance of thorax - should be compared. Should the male flies from the diets be tested and compared by means of the Sexual Aggressiveness Test? Are there other variables that you would suggest?

Your comments will be appreciated.

Ray

R. F. Moore, Jr.
Research Entomologist
SE Cotton Insects Investigations

cc:
R. Gingrich
W. Klassen

B

1 OCT 1973

UNITED STATES DEPARTMENT OF AGRICULTURE

AGRICULTURAL RESEARCH SERVICE

Southern Region

Georgia-South Carolina Area

Southeastern Cotton Insects Investigations

P. O. Box 271, Florence, S. C. 29501

September 25, 1973

Subject: Nutritional Experiments with the Screwworm Fly

To: R. C. Bushland, Research Leader
Lab. for Screwworm Research, Mission, Texas

Through: H. M. Taft, Research Leader
SE Cotton Insects Investigations

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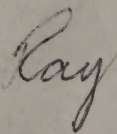
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R. F. Moore, Jr.
Research Entomologist
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R. Gingrich
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What will APHIS / Juncos

Dec 14/15

R. E. Moore, Jr.
Research Entomologist
SE Cotton Insect Investigations

LSWY

cc:
R. Gieseler
W. Klassen

Wm. J. Juncos

504-527-6914

Ray Moore's Report Dec 13, 1973

Dr. R. E. ^Hgingrich and I were requested to review the nutrition of the screwworm in a memorandum from Dr. Waldmar Klassen, dated Feb. 26, 1973. Dr. Gingrich and I visited the Screwworm Research Laboratory, Mission, Texas in early April 1973 and plans were made to conduct a series of experiments, to begin in the fall, and last approximately one month. The experiments were to be designed to produce, from an ^{laboratory} ~~artificial~~ diet, a fly with the "blue" color and "broad" thorax of the wild or animal reared fly.

To assist in the ~~d~~experimental design, information on the composition of beef blood, amino acid content of blood and muscle, and an amino acid analysis of screwworm larvae and adults was requested. Dr. Klassen assisted in routing ~~this~~ request to the ~~per~~ proper individuals so that the data was available for the experiments.

I arrived in McAllen, Tex. on October 28, 1973 and reported to the Screwworm Research Laboratory on Monday October 29. Dr. Gingrich ~~was~~ also there and after consultation with Dr. R. C. Bushland and Jack Graham, a series of experiments was selected.

EXPERIMENTAL DESIGN

The available data indicated that a "wild" type of fly had not been produced, even on the best diets of horse meat, blood, and plasma. Thus, it seemed advisable to attempt to make changes in this ~~best~~ "best" diet to determine if it could be modified to produce a "wild" type of fly.

Examination of the experiments with horse meat: blood: plasma diets revealed that the blood or plasma was always diluted with water by a factor of 1/3 to 1/2.

The reasons for the limitation on color, robustness, and larval size ~~in~~ ~~laboratory~~ of insects from laboratory media are not known. They may be related to the differences between "living" and "dead" tissue; such differences

would be subtle, perhaps complex and difficult to determine. It was felt that a deficiency in some major component of the media, such as protein, minerals or vitamins, should be eliminated as a first step and the primary design of the experiments was to determine if dilution of one ~~of these~~ or all of these components limited the type of fly produced from the diets. Availability of food, the accumulation of wastes, and pH were also recognized as possible limitations on the type of fly and some experiments were designed to test these factors.

← EVALUATION OF RESULTS

The original intention was to observe the incidence of "blue" flies and make some measurement of the thorax as an indication of the success of the ~~diff~~ modifications of the diet. It was soon apparent that there was considerable variation in the color and appearance in both lab and wild flies and that valid objective measurements would be difficult. ^{to find} Discussions with individuals associated with the Screwworm Project raised several questions about the color of wild flies and the factor(s) that make the wild fly appear more "broad shouldered" or robust. The incidence of "green" screwworms in nature, the size variation and the effect of pre-mature pupation (forced by death of the host) on color and robustness of the flies are ~~a few of~~ ^{other} the unknowns.

Some individual felt that there was a color change in the flies after death. This was tested by freezing some Laboratory reared flies, then holding some at room temperature, heating some at 100° F for several hours and putting others in the sun. Those heated at 100° F. and exposed to the sun changed to the blue color of the wild fly. Flies which are caught in traps might be exposed to such conditions either in the trap or in the vehicle in which they are transported. Thus, an unknown number of ~~wild~~

green wild flies may change to blue in the trapping and handling process. Captured laboratory flies might also turn blue and be incorrectly identified as wild.

The factors that constitute the "broad" thorax and contribute to the "robust" appearance of the wild fly were difficult to define. Dr. Bill Hightower had, in the past, make measurements of the width and length of the thorax of wild and laboratory reared flies. The ratio of length/width was found to be 1.02 ± 0.02 for 25 $\bar{x}$ wild flies and 1.05 ± 0.02 for 25 lab flies. This is not statistically significant by the t test. Efforts to find specific characteristics to measure the "robust" characteristic were unsuccessful.

The robust characteristic was thought to be present, even in small wild flies which result from premature pupation. This was discussed with ~~Mr.~~ Mr. Calvin Jones and at his suggestion a field trip was made to the King Ranch at Encino, Tex. and larvae were obtained from the ears of cattle. A group of larvae were forced to pupate without complete feeding and produced pupae weighing ~~46~~ 49 mg. This corresponds to a larval weight of approximately 70 mg and would be slightly larger than the usual laboratory reared larvae. A second group of larvae were about 24 hours old; these were forced into the horse meat diet where development was completed and pupae weighing 53.9 mg were produced. Mr. Jack Graham examined the adult flies from these larvae and compared them with flies that had been reared on sheep, on hydroponic medium and horse meat medium. Only those from sheep had the appearance of wild flies. Thus, flies which had developed from larvae taken from cattle and forced to pupate prematurely or which completed a substantial portion of their larval development on laboratory medium could not be distinguished

from flies reared on media. In other words, both blue and green flies were present and they did not have the robust appearance of wild flies. Therefore, it was concluded that the "blue" color and broad thorax or robust appearance were inadequate for evaluation of differences in flies from the experimental diets.

Since the original characteristics were lacking in objectivity, the following five methods were selected for evaluation of the flies from the experimental diets. (1) The weight of 10 larvae which were selected just prior to pupation. (2) Three technicians, experienced in the classification of "wild" and "Medium" reared flies were asked to classify 20 ^{males} flies from each diet. A successful diet should show a larger percentage of "wild" type flies. (3) Flies which have been in the laboratory for a number of generations are more active and appear to have a different type of movement than wild flies, so observations were made to determine if there were any behavioral differences in the flies from different diets. (4) Dr. M. M. Crystal has modified (for the screwworm) a published method for determining the tendency of an insect to fly. This method was applied to the males from each diet in the expectation that flies from the best diets might have a greater tendency to fly. (5) Jack Graham had done some experiments with ~~the effect of exposure of adult flies to 102° F~~ survival of adult flies at ~~a temperature~~ 102° F. It was reasoned that survival under this type of stress would be a desirable characteristic and such flies should be found in the best diets. In the test, 20 flies that were 5 days old were exposed to 36° C without food or water of 16 hours and the percent ^{Mortality} ~~survival~~ recorded. ^{should be} The value of the "flight" and "stress" tests ~~was~~ examined by comparing adult flies that developed from animal hosts with laboratory reared flies of the same age.

Examination of the results of the evaluation tests of flies from the Group III diets suggested the possibility that the size of the flies might be a factor in the survival under the stress of 95° F for 16 hours, without food or water. Therefore, some measurements of the wet weights of survivors were made immediately at the end of the test. Comparison of the weights of the survivors and dead flies was accomplished by drying both groups at 95°F for 24 hours after both were killed and their weights were constant.

EXPERIMENTAL PROCEDURE AND TEST DIETS

Five groups of experimental diets were set up. Each group contained a Standard diet which contained 1000 ml water, 500 ml blood plasma, and horsemeat ^{4 ml HCHO} to produce a proper consistency (approximately 1500 gm).

Unless otherwise indicated, the changes in the diets were to modify the 1000 ml of water by adding egg albumin, minerals, vitamins or by substitution of plasma for the water.

The diets were made up on Fri. afternoon and one ~~1/1~~ replicate of each diet was started on Sat., Sun., and Mon. The larvae pupated in 4 days and emerged as adults in 15 days. The adults were evaluated when 5 days old.

~~on Sat., Sun., and Mon. Larvae pupated on Wed., Thurs., ~~7/7~~ and Fri.;~~
~~and emerged as adults one week later. Adults were evaluated when 5 days old.~~

The diets were:

Group I*

1. Standard
2. 0.1% Wesson salts
3. 0.1% Human blood salt mix
4. 0.1% Salt mix by House
5. 20 ml of Vanderzans vitamin mix

* Dr. Gingrich determined the osmotic pressure of the salts after the tests were begun and found that 0.9% was more nearly isotonic. It was also found that the vitamins were only 1/3 of that which might be required. Thus, flies from this group ~~several~~ were used in an effort to standardize the evaluation procedures.

Group II

1. Standard
2. Undiluted plasma
3. 6% Egg albumin + amino acids*
4. 6% Egg albumin + amino acids, 0.1% Wesson salts, 20 ml. vitamins,
5. 6% Egg albumin
6. 6% Egg albumin + amino acids, 5% dried blood, 0.1% Wesson salts, 20 ml. vitamin mix.

*Selected amino acids were added to the egg albumin to make its amino acid composition more like that of bovine plasma.

Group III

1. Standard - pH not adjusted
2. Standard - pH 7.0
3. 1000 ml citrated whole blood, pH not adjusted
4. 3% Egg albumin, pH 7.0
5. 3 % Egg albumin, + Lysine and Phenylalanine,* pH 7.0
6. 3% Egg albumin, + Lysine and Phenylalanine*, pH not adjusted

* The "protein score" concept was used as the basis for modifying the amino acid composition of the egg albumin. The carcass analysis of larvae reared on sheep was used as the standard and lysine and phenylalanine were the most limiting amino acids.

Group IV - all diets were pH 7.0

1. Standard
2. 0.9% Wesson salt mix
3. 0.9% Human blood salt mix
4. 0.9% House salt mix
5. 60 ml., Vanderzant vitamin mix
6. 3% Egg albumin, 60 ml vitamin mix, 0.9% Human salt mix.

Group V - all diets were pH 7.0

1. Standard
2. 6% Egg albumin
3. 6% Whole egg
4. 12% Cottonseed flour
5. Texturized soybean protein, ^(No) 6% egg albumin, 0.9% Human salt mix, 60 ml vitamin mix, 500 ml plasma, no horse meat
6. Texturized soybean protein, 1000 ml of a ~~11~~ 1:1 dilution of hydroliquid, 500 ml plasma, no horse meat.

RESULTS:

Group II diets; Substitution of plasma for the water and the addition of egg albumin in the water of dilution produced a slightly larger larvae (Table 1, Diet 2) than *did* the Standard diet (Table 1, Diet 1). Flies from Diet 3 were the least responsive in the flight test (Table 2) and had one of the higher mortalities in response to Stress (Table 3). Flies from Diet 6 were most responsive in the flight test (Table 2) and had one of the lower stress mortalities (Table 3).

Group III diets: Adjustment of the pH to 7.0 did not make any consistent difference in larval weights, flight response, or stress response (Tables 1, 2, 3). Addition of lysine and phenylalanine to egg albumin (Diets 5 & 69) to correct a supposed deficiency of these amino acids (based on amino acid analysis of the carcass of larvae which had developed on sheep) did not produce any change in larval weight (Table 1) or flight response (Table 2) but flies from this diet at ~~pH 7.0~~ at pH 7.0 did have a higher stress mortality (Table 3)

Flies from the Mass rearing Plant were tested with Groups II and III. They were less responsive in the flight test and had a higher stress mortalities than those from the experimental diets.

Group IV diets: This group of diets were expected to be the most significant of those tested because the media was adjusted to pH 7.0, the concentrations of ~~vitamins~~ vitamins and minerals were adjusted ~~over~~ to a more nearly correct amount than those in Group II, and ~~the~~ modifications in the techniques of handling the larvae and diets had been made. Examination of the larval weights, flight tendency, and stress response does not show any obvious differences between the Standard and the test diets.

Group V diets: These diets were to test the quality of different proteins. The larger larval weights in this group are attributed to the reduced number of larvae per container and the addition of plasma to make the diet more liquid

during the last 24 hours. Diets 1, 2, and 3 are comparable. Diet 4 with 12% cottonseed flour^(CSF) did not support growth beyond 24 hours, reduction to 6%^(CSF) at the end of 24 hours improved growth somewhat and a further reduction to 3%^(CSF) at the end of 48 hours permitted the larvae to mature. The cottonseed flour may have some value in diets at this level. Diets 5 & 6 did not support growth. the texturized soybean protein was substituted for the meat and constituted approximately 50% of the diet.

Examination of some of the Flight tendency and Stress response data gives an impression that a low tendency to flight is associated with a high stress mortality. For example: Group II, diet 3 (Table 2) show a low flight response and higher stress mortality (Table 3) than the experimental diets. However, a plot of all the data does not show such a relationship.

The weights of surviving and dead flies were determined in a few of the later tests. Both survivors and dead were dried for 24 hours at 95° F. It was found that the survivors with few exceptions weighed more than the dead flies (Table 4). This was true in 13 of 17 which were compared.

The wet weight of the survivors in Group IV were determined (Table 5). It is seen that the survivors average about 25 mg regardless of diet or replicate.

The data were examined further by plotting Flight response against larval weight (Fig 1). The distribution of the points suggests that the larger flies have a greater tendency to fly. Perhaps additional data and refinement of the test method would produce a clearer relationship.

A similar plot of Stress Mortality vs Larval weight indicates that the larger flies have the least mortality. Again additional data and refinement of the test might establish a clearer relationship.

The conditions in a wound were simulated by the apparatus in Fig. 3. It provides for a continuous flow of plasma over the larvae to remove the waste products and supply fresh nutrients. In one set-up, cotton linters were substituted for meat and ^{the} plasma was the only source of nutrients. One group ^{956 mg} of larvae presumed to come from the meat weighed 84.8 mg; a second group 90 mg. Those with cotton linters only weighed 50 mg.

SUMMARY

1. Adult flies will turn blue ^{after death} when exposed to temperatures near ~~199~~ 95° F.
2. Flies reared on~~ly~~ an animal and forced to pupate prematurely cannot be distinguished from media reared flies.
3. Fresh meat diets and Fresh Hydro diets have a pH of 6.0. Diet 24 hours old and "spent" Hydro have a pH of about ~~6~~ 7.0. Based on~~ly~~ larval weights, adjustment to a pH of 7.0 of the meat diets did not seem to make in difference in their growth (Group III diets).
4. Only the addition of protein seemed to increase larval size. No other change ~~seemed to~~ in the diet seemed to make any difference. There seems to be a limit, imposed by some other factor^(s); Factors such as the effect of waste products or the availability of food (especially during the last 24 hours).
5. Flies from large larvae appear to have the highest response in the Flight tendency test. A high response and large size may indicate greater "biological vigor". (Table 2) and Fig. 1
6. ~~Exposure to heat/stress~~ The largest flies survive exposure to the stress of 16 hours exposure to 95° without food ~~or~~ water. There also appears to be a correlation between larval weights and stress survival (Fig. 2)
7. The findings in 7, and 8 and from Figs 1 & 2 suggest that ~~stress/survival~~ flight response and stress survival are much lower from larvae ~~with a size~~

weighing less than 65mg than it is for larvae which weigh more.

MISCELLANEOUS

1. The question of the toxicity of formaldehyde has been raised on several occasions. If it is toxic, it may be due to its formic acid content. This might be checked and if formic acid is ~~toxic~~ toxic, the formaldehyde should be stored over calcium chips.
2. It was noted that benzene is used as a killing agent in the traps. This is one of the most toxic of the organic solvents. It is suggested that Ethyl Acetate be tried as a substitute.
3. ~~One observation of animal~~ One comparison of wild animal reared flies with those reared in the laboratory seemed to show a different type of activity and movement between the two sources. Those from the lab. seemed ~~hyper~~ hyperactive with movements that were jerky and excessive. There seemed to be less waste movement in the wild flies. Other observations have not confirmed this but additional observations are suggested; if confirmed the movement may indicate a deficiency of ~~either~~ thiamine and/or niacin.

ADDITIONAL STUDIES:

I Response to Stress

1. Refine test and control variables more closely
 - a. Control of age of fly
 - b. Pre-Treatment conditions of feeding, holding etc.
2. Response of flies from different size larvae from a single, standard diet to establish a weight:Mortality curve.
- 2a. *Rel. resp. of lab stress to survival in cages in nature*
3. Begin with wild flies and determine if a change in stress response occurs through successive generations.

II Flight Tendancy

1. ~~Attempt~~ Attempt modification of test container
 - a. More dependable butterfly valve
 - b. Capture device for those animals that fly, so they can be kept alive.
2. ~~Follow~~ Follow Flight response through successive generations and in I, 3 above.

III Diet experiments

1. Better control of variables in the experiments with horsemeat
 - a. Number of larvae/jar (100-200)
 - b. Change of food (intervals)
 - c. Amount of liquid in diet
 - d. Better temperature control (Closer watch on water level)
3. Elimination of waste (Appears to be a limiting factor)
 - 1) Microbial inhibitors
 - 2) Effect of larval excretion (esp. last 24 hr.)

III 2. If the waste products is determined to be the limiting factors, then

it might be desirable to repeat the sequence of diets and tests.

IV Stress response of flies from vasts with 3, 4, 5, 6, 7 gms of eggs *to produce a blue & robust wild fly* ~~Frank D~~
& flight resp.

Many individual contributed much to this research, both in actual assistance in the work and in discussions of the varied aspects of the screwworm program. Dr. Bushland, Dr. Gingrich, Jack Graham and Frank Dutley were especially helpful in acquainting me with the screwworm program and providing basic information on the various aspects of rearing screwworms. Dr. Bushland, in addition, provided the necessary materials and facilities for the research. ~~W~~ Thelma Jester was also helpful in securing materials and assistance. I graatly appreciate the willingness of Dr. M. M. Crystal to permit the free use of his laboratory spae and equipment. It isn't always easy to adust one's research program ~~by~~ the temporary needs of a vistor and I hope my presence hasn't been too great an inconvenience.

The services of Bob ~~H~~^{Hadke} are much appreciated. Bob was dependable in carrying out instructions, performed his duties with a minimum of instruction, he anticipated the needs of the program, he showed ing~~g~~enuity in adapting laboratory apparatus to different needs and willingly worked additional hours outside of the normal work ~~sh~~^{his} schedule. I beleive he is an excellent technician and should be encouraged to further ~~his/normal~~ increase his technical and formal training.

TABLE 1

LARVAL WEIGHTS

DIET		REPLICATE			
<u>Group II</u>					
	Diet	1	2	3	Average
Standard	1	65.8		69.6	67.7
1500 plasma	2	70.4		75.4	72.9
6% EA + AA	3	72.0		70.0	71.0
6% EA + AA WS, VM	4	68.7		63.9	66.3
6% EA	5	77.9		74.6	76.3
6% EA, WS 5% DB, VM SHEEP	6	56.0		58.1	57.1
		95.0 (pupae-78 gm)			
<u>Group III</u>					
Standard, pH Na	1	72.5	70.7	65.8	69.7
Standard pH 7.0	2	71.8	71.1	69.0	70.6
Whole Blood pH Na	3	64.0	49.3	—	—
3% EA pH 7.0	4	72.2	69.3	66.7	69.4
3% EA + Ly, PH pH 7.0	5	68.9	68.6	66.7	68.1
3% EA + Ly, Ph pH Na	6	71.4	74.0	62.7	69.4

LARVAE FROM APPARATUS WITH PLASMA EXCHANGE

Gr. III - 68 mg vs 69.7 (Larvae); Regular); 53.7(flow) vs 50.8(Reg.)- pupae

Gr. IV 51.5(flow) vs 53.5(Reg.)-pupae

FUNNEL (meat) 84.8mg

" " 90 - Largest 95 mg

TABLE 2

FLIGHT - 3 replicates of 20 males each unless indicated otherwise

(Percent responding)

DIET		REPLICATE			
		Percent responding			
<u>Group II</u>	Diet	1	2	3	Average
Standard	1	45 (9/20)		33 (13/40)	39
1500 plasma	2	45 1/20		32	39
6% EA + AA	3	40		24	32
6% EA + AA	4	38		32	36
WS, VM					
6% EA	5	37		45	41
6% EA, WS	6	53		48	51
5% DB, VM					
Plant		20 (14/40)	33 (13/40)		27
Sheep		35 (7/40)			35
<u>Group III</u>					
Standard,	1	47	23	30	33
pH Na					
Standard	2	55	23	17	32
pH 7.0					
Whole Blood	3	23	—	—	—
pH Na					
3% EA	4	51	44	23	39
pH 7.0					
3% EA + Ly, PH	5	42	42	45	43
pH 7.0					
3% EA + Ly, Ph	6	43	48	33	41
pH Na					
Plant		43 (7/40)	27	—	35

FLIGHT

DIET		REPLICATE			
<u>Group IV</u>					
	Diet	1	2	3	Avera ge
Standard	1	20	48.4	38.4	35.6
8.9% WS	2	15	41.8	47.1	34.6
0.9% HB	3	13.3	41.8	46.7	34.1
0.9% Ho	4	30	41.8	-	35.9
VM	5	18.3	36.7	46.7	33.9
3% EA, VM 0.9% HB	6	25	38.4	-	31.7

<u>Group V</u>	
Standard	7 1
6.8% EA	2
6.8% Whole egg	3
12% Cottonseed flour	4
Textured Soybean protein, 6% EA	5
Tex. Soybean protein, 50% Hydro.	6

Abbrev.

EA - Egg albumin
DB - Dried Blood
VM - Vanderzant Vitamin
mix

AA - Amino Acids
HB - Human Blood Salt

WS - Wesson Salt
Ho - House Salt

TABLE 3

STRESS *- 20 males unless indicated otherwise

DIET	REPLICATE				
	Percent Mortality				
<u>Group II</u>	Diet	1	2	3	Average
Standard	1	90 ^{1/2}		35	62.5
1500 plasma	2	85 ^{1/2}		100	92.5
6% EA + AA	3	75		100	87.5
6% EA + AA WS, VM	4	60		90	85
6% EA	5	30		89	59.5
6% EA, WS 5% DB, VM	6	55		70	62.5
Plant		100			100
Sheep		40 ^{1/2}	55 ^{1/2}		40
<u>Group III</u>					
Standard, pH Na	1	0?	60	100	80
Standard pH 7.0	2	15?	75	70	72.5
Whole Blood pH Na	3	75	-	-	75
3% EA pH 7.0	4	75	85	40	66.7
3% EA + Ly, pH pH 7.0	5	90	95	95	93.3
3% EA + Ly, Ph pH Na	6	65	75	65	68.3
Plant		100	90	-	95

STRESS

DIET

REPLICATE

<u>Group IV</u>	Diet	L	2	3	Average
Standard	1	90	100	60	83.3
0.9% WS	2	95	95	95	95
0.9% HB	3	60	85	95	80
0.9% Ho	4	100	100	100	100
VM	5	95	100	100	97
3% EA, VM	6	80	85	-	82.5
0.9% HB PLANT-TxMx		65	40		52.5

Group V

Standard	1
6 3% EA	2
6 3% Whole egg	3
12% Cottonseed flour	4
Textured Soybean protein, 6% EA	5
Tex. Soybean protein, 50% Hydro.	6

Abbrev.

EA - Egg albumin
 DB - Dried Blood
 VM - Vanderzant Vitamin mix

AA - Amino Acids
 HB - Human Blood Salt

WS - Wesson Salt
 Ho - House Salt

DRY WEIGHTS OF SURVIVORS AND DEAD OF HEAT STRESS

DIET

REPLICATE

Group IV

		1		2		3		Average	
		Survivors	Dead	Survivors	Dead	Surviv.	Dead	Surviv.	Dead
1	Standard	9.9	9.1		9.9	9.9	9.4		
2	0.9% WS	9.0	8.2	8.7	8.3	9.0	8.7		
3	0.9% HB	8.5	8.0	8.6	8.8	8.0	8.5		
4	0.9% Ho	-	7.8		9.3		9.0		
5	VM	9.7	8.7		9.0		19.0		
6	3% EA, VM	8.7	8.9		9.1				
	0.9% HB								
	Plant(TxMx)	8.7	8.5	9.3	8.4				
	AVERAGE	9.1	8.4						

Group V

1	Standard
2	6.8% EA
3	6.8% Whole egg
4	12% Cottonseed flour
5	Textured Soybean protein, 6% EA
6	Tex. Soybean protein, 50% Hydro.

Abbrev.

EA - Egg albumin
 DB - Dried Blood
 VM - Vanderzant Vitamin mix

AA - Amino Acids
 HB - Human Blood Salt

WS - Wesson Salt
 Ho - House Salt

WET
WEIGHT OF MALES SURVIVING HEAT STRESS

DIET		REPLICATE			
<u>Group IV</u>					
	Diet	1	2	3	Average
Standard	1	29.1	27.1	22.1	25.6
0.9% WS	2	26.9	25.8	27.6	26.8
0.9% HB	3	23.1	24.8	22.7	23.5
0.9% Ho	4	—	—	—	—
VM	5	25.1			25.1
3% EA, VM	6	25.6	25.2		25.4
0.9% HB					
PLANT-TxMx		23.2	25.1		24.1
	Av.	<u>25.5</u>	<u>25.2</u>	<u>24.1</u>	

Group V

Standard	1
63% EA	2
63% Whole egg	3
12% Cottonseed flour	4
Textured Soybean protein, 6% EA	5
Tex. Soybean protein, 50% Hydro.	6

Abbrev.

EA - Egg albumin
 DB - Dried Blood
 VM - Vanderzant Vitamin mix

AA - Amino Acids
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WS - Wesson Salt
 Ho - House Salt

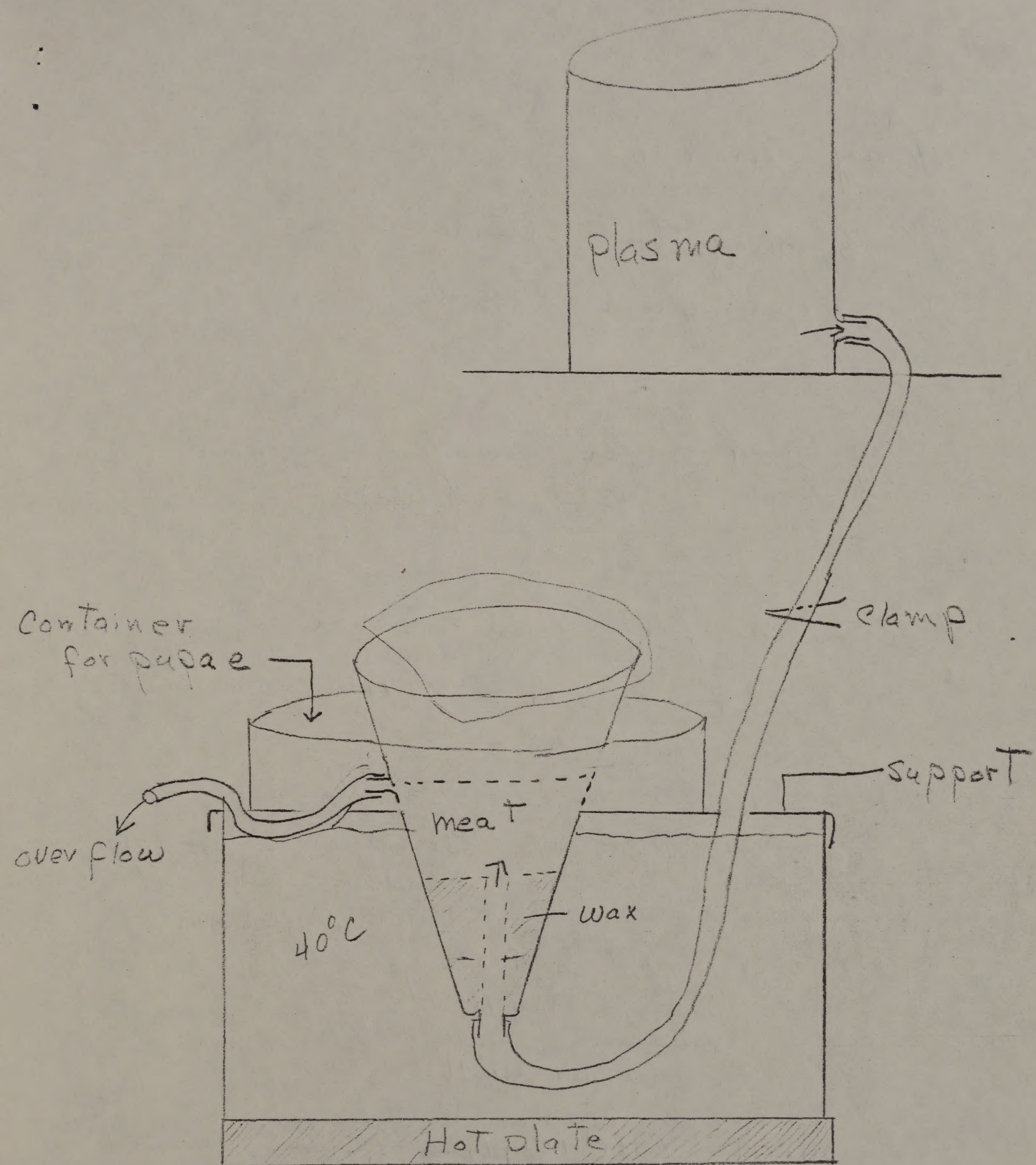


Fig. 3

Ray Moore —

1. 3 magnetic stirrers —
2. 5 gal fresh blood —

Guy Bush = for executive conference —
22nd, 23rd

Agenda for Tues. afternoon

Begin at 11:00 AM — 12:00 noon Bush history
lunch — at lab —

Methods Develop. — about 1 hr.
a. attractants Research — Nightower
Field Tests — Jones — Graham — logistics

Interpretation
of
data

→ Crystal — ~~physio~~ tour lab — Wed AM —
genetic strains —
Guy Bush —
nutrition — Ray Moore —
progress report

~~at~~ Coop. agreement research —
needed Field Work — {genetic strains
field evaluation

Friday — Draft of agenda — other ecology

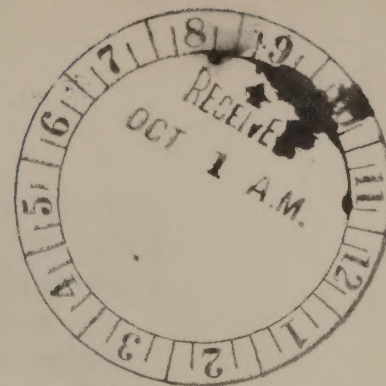
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NASA coop. —

Thebman
F. L.

Dir. Wh. Hen
Oct 1 Return

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Southern Region
Georgia -South Carolina Area
Southeastern Cotton Insects Investigations
P. O. Box 271, Florence, S. C. 29501



January 9, 1974

Subject: Report of Nutritional Research Conducted on Screwworm,
Oct. 29, 1973 to Dec. 12, 1973, at Mission, Texas

To: W. M. Bruce, Tifton, Ga. Lyman Henderson, Beltsville, Md.
R. C. Bushland, Mission, Tex. Chris Hoffman, Mission, Tex.
E. Corristan, Mission, Tex. C. H. Hoffman, Beltsville, Md.
M. M. Crystal, Mission, Tex. W. Klassen, Beltsville, Md.
H C Cox, New Orleans, La. E. F. Knipling, Beltsville, Md.
R. E. Gingrich, Kerrville, Tex. M. E. Meadows, Mission, Tex.
Jack Graham, Mission, Tex. E. A. Taylor, Weslaco, Tex.

Through: H. M. Taft, Research Leader

The subject report is the result of research conducted during a temporary assignment to the Screwworm Research Laboratory, Mission, Texas.

The original goal of producing a wild type of fly with a blue color and robust appearance was not accomplished, but the more general goal of determining if some nutritional changes in the rearing might produce a better quality fly was realized. The results of the studies indicated (1) that the waste products from the larvae probably limits their size, (2) that the lower limits on the size of acceptable larvae should be 65-68 mg instead of the present 60 mg, and (3) that larval size and the mortality resulting from exposure of adult flies to heat stress could give laboratory data that would have a direct relationship to survival in the field and consequently the performance of the released flies in the eradication program.

Some additional experiments will be conducted in January and February 1974 to complete portions of the studies.

R. F. Moore, Jr.

[Signature]

R. F. Moore, Jr.
Research Entomologist

Dr. R. E. Gingrich and I were requested to review the nutrition of the screwworm in a memorandum from Dr. Waldemar Klaseen dated Feb. 26, 1973. Dr. Gingrich and I visited the Screwworm Research Laboratory, Mission, Texas in early April 1973 and plans were made to conduct a series of experiments, to begin in the fall and lasting approximately one month. The experiments were to be designed to produce, from a laboratory diet, a fly with the "blue" color and "broad" thorax of the wild or animal-reared fly.

REPORT OF NUTRITIONAL STUDIES ON THE SCREWORM
CONDUCTED AT THE SCREWORM RESEARCH LABORATORY

To assist in the experimental design, information on the composition of beef blood, and amino acid analysis of screwworm larvae and adults was requested. Dr. Klaseen assisted in routing this request to the proper individuals so that the data were available for the experiment.

Mission, Texas

October 29 - December 12, 1973

I arrived in Mission, Texas on Oct. 29, 1973 and reported to the Screwworm Research Laboratory on Monday, Oct. 29. Dr. Gingrich was also there and after consultation with Dr. R. C. Bushland and Jack Graham, a series of experiments was planned.

The available data indicated that a "wild" type of fly had not been produced, even on the best diets of horse meat, blood and plasma. Thus, it seemed advisable to attempt to produce the "blue" fly. Examination of the experiments with the "blue" fly revealed that the blood or plasma was always diluted with water by a factor of 1/3 to 1/2.

by
R. F. Moore, Jr., Research Entomologist
Southeastern Cotton Insects Investigations
Florence, S. C. 29501

The reasons for the limitation on color, robustness, and larval size of insects from laboratory diets are not known. They may be related to the differences between "living" and "dead" tissue; such differences would be subtle, perhaps complex and difficult to determine. It was felt that a deficiency in some major component of the media, such as protein, minerals or vitamins, should be eliminated as a first step. Therefore, protein as egg albumin, mineral mixtures, or a vitamin mixture were added to the 1000 ml of water and in addition, plasma was substituted for the 1000 ml of water to determine if dilution of one or all of these components determined the type of fly produced from the diets. Availability of food, the accumulation of wastes, and pH were also recognized as possible limitations on the type of fly and more experiments were designed to test these factors.

The original intention was to observe the incidence of blue flies and make some measurements of the thorax as an indication of the success of the modifications of the diet. It was soon apparent that there was considerable variation in the color and appearance in both lab and wild flies and that valid objective measurements of the differences would be difficult. Discussions with individuals associated with the Screwworm Project raised several questions about the color of wild flies and the factor(s) that make the wild fly appear more "broad shouldered" or robust. The incidence of "green" screwworm flies in nature, the size variation and the effect of premature pupation (forced by death of the host) on color and robustness of the flies are other unknowns.

Dr. R. E. Gingrich and I were requested to review the nutrition of the screwworm in a memorandum from Dr. Waldemar Klassen dated Feb. 26, 1973. Dr. Gingrich and I visited the Screwworm Research Laboratory, Mission, Texas in early April 1973 and plans were made to conduct a series of experiments, to begin in the fall and lasting approximately one month. The experiments were to be designed to produce, from a laboratory diet, a fly with the "blue" color and "broad" thorax of the wild or animal-reared fly.

To assist in the experimental design, information on the composition of beef blood, amino acid content of blood and muscle, and an amino acid analysis of screwworm larvae and adults was requested. Dr. Klassen assisted in routing this request to the proper individuals so that the data were available for the experiments.

I arrived in McAllen, Texas on Oct. 28, 1973 and reported to the Screwworm Research Laboratory on Monday, Oct. 29. Dr. Gingrich was also there and after consultation with Dr. R. C. Bushland and Jack Graham, a series of experiments was planned.

The available data indicated that a "wild" type of fly had not been produced, even on the best diets of horsemeat, blood and plasma. Thus, it seemed advisable to attempt to make changes in the "best" diet to determine if it could be modified to produce a wild type fly. Examination of the experiments with horsemeat: blood: plasma diets revealed that the blood or plasma was always diluted with water by a factor of 1/3 to 1/2.

The reasons for the limitation on color, robustness, and larval size of insects from laboratory diets are not known. They may be related to the differences between "living" and "dead" tissue; such differences would be subtle, perhaps complex and difficult to determine. It was felt that a deficiency in some major component of the media, such as protein, minerals or vitamins, should be eliminated as a first step. Therefore, protein as egg albumin, mineral mixtures, or a vitamin mixture were added to the 1000 ml of water and in addition, plasma was substituted for the 1000 ml of water to determine if dilution of one or all of these components determined the type of fly produced from the diets. Availability of food, the accumulation of wastes, and pH were also recognized as possible limitations on the type of fly and some experiments were designed to test these factors.

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Some individuals felt that there was a color change in the flies after death. This was tested by freezing some laboratory-reared flies, then holding some at room temperature, heating some at 37°C for several hours, and putting others in the sun. Those heated at 37° or exposed to the sun changed to the blue color of the wild fly. Flies which are caught in traps might be exposed to such conditions either in the trap or in the vehicle in which they are transported. Thus, an unknown number of green wild flies may change to blue in the trapping and handling process. Captured laboratory flies might also turn blue and be incorrectly identified as wild.

Dr. Bill Hightower had, in the past, made measurements of the width and length of the thorax of wild and laboratory-reared flies. The ratio of length/width from these data was found to be 1.02 ± 0.02 for 25 wild flies and 1.05 ± 0.02 for 25 lab flies. This is not statistically significant by the t test. Efforts to find specific characteristics to measure the "robust" characteristic were unsuccessful.

The robust characteristic was thought to be present, even in small wild flies which result from premature pupation. This was discussed with Mr. Calvin Jones and at his suggestion a field trip was made to the King Ranch at Encino, Texas. Larvae in the ears of cattle were obtained; a group of large larvae were forced to pupate without completing their feeding and produced pupae weighing 49 mg. This corresponds to a larval weight of wild flies of approximately 70 mg but would be slightly larger than the usual laboratory-reared larvae. A second group of larvae was about 24 hours old; these were forced into the horse meat diet where development was completed and pupae weighing 53.9 mg were produced. Mr. Jack Graham examined the adult flies from these larvae and compared them with flies that had been reared on sheep, on hydroponic diets, and horse meat diets. Only those from sheep had the appearance of wild flies. Thus, flies which had developed from larvae taken from cattle and forced to pupate prematurely or which completed a substantial portion of their larval development on laboratory diets could not be distinguished from flies reared wholly on laboratory diets. In other words, both blue and green flies were present and they did not have the robust appearance of wild flies. It was concluded that the blue color and broad thorax or robust appearance were difficult to define and were inadequate for evaluation of differences in flies from the experimental diets.

Since the original characteristics were lacking in objectivity, the following methods were selected for evaluation of the flies from the experimental diets: (1) Average larval weight as determined by the weight of 10 larvae which were selected just prior to pupation; (2) Tendency to fly-- this was a modification of a published method for determining the tendency of an insect to fly. Modification of the method, equipment, and initial testing had been done by Dr. M. M. Crystal. This method was applied to the males from each diet in the expectation that flies from the best diets might have a greater tendency to fly; (3) Stress survival-- Jack Graham had done some experiments with the survival of adult flies at 39°C. It was reasoned that survival under this type of stress would be a desirable characteristic and such flies should be found in the best

diets. In the test, 20 male flies that were 5 days old were exposed to 36°C, without food or water for 16 hours and the percent mortality recorded; (4) Categorizing by physical characteristics-- 3 technicians, experienced in the classification of wild and laboratory-reared flies, classified 20 male flies from each diet. A successful diet should show a larger percentage of flies appearing like the wild type.

Examination of the results of the evaluation tests of flies from the Group III diets suggested the possibility that the size of the flies might be a factor in survival under the stress of exposure, without food or water, to a temperature of 36° for 16 hours. Therefore, some measurements of the wet weights of survivors were made immediately at the end of the test. Measurements of the dry weights of the survivors and dead flies were made after drying to a constant weight at 36°C.

Five groups of experimental diets were set up. Each group contained a Standard diet which contained 4 ml formaldehyde, 1000 ml water, 500 ml blood plasma, and macerated horse meat (approximately 1500 gm) for a proper consistency. Unless otherwise indicated, the changes in the diets were to modify the 1000 ml of water by adding egg albumin, minerals, vitamins or by substitution of plasma for the water.

The diets were made up on Friday afternoon and one replicate of each diet was started on Sat., Sun., and Mon. The larvae pupated in 4 days and emerged as adults in 15 days. The adults were tested when 5 days old.

The diets were:

Group I*

1. Standard
2. 0.1% Wesson salts
3. 0.1% Human blood salt mix
4. 0.1% House salt mix
5. 20 ml of Vanderzant vitamin mix

* Dr. Gingrich determined the osmotic pressure of the salts after the tests were begun and found that 0.9% was more nearly isotonic. It was also found that the vitamins were only 1/3 of that which might be required. Thus, flies from this group were used in an effort to standardize the evaluation procedures.

Group II

1. Standard
2. Undiluted plasma
3. 6% egg albumin + amino acids*
4. 6% egg albumin + amino acids, 0.1% Wesson salts, 20 ml vitamins
5. 6% egg albumin
6. 6% egg albumin + amino acids, 5% dried blood, 0.1% Wesson salts, 20 ml vitamin mix

* Selected amino acids were added to the egg albumin to make its amino acid composition more like that of bovine plasma.

Group III

1. Standard - pH not adjusted
2. Standard - pH 7.0
3. 1000 ml citrated whole blood, pH not adjusted
4. 3% egg albumin, pH 7.0
5. 3% egg albumin + Lysine and Phenylalanine*, pH 7.0
6. 3% egg albumin + Lysine and Phenylalanine*, pH not adjusted

* The "protein score" concept was used as the basis for modifying the amino acid composition of the egg albumin. The carcass analysis of larvae reared on sheep was used as the standard and lysine and phenylalanine were the most limiting amino acids.

Group IV - all diets were pH 7.0

1. Standard
2. 0.9% Wesson salt mix
3. 0.9% Human blood salt mix
4. 0.9% House salt mix
5. 60 ml Vanderzant vitamin mix
6. 3% egg albumin, 60 ml vitamin mix, 0.9% Human salt mix

Group V - all diets were pH 7.0

1. Standard
2. 6% egg albumin
3. 6% whole egg
4. 12% cottonseed flour
5. Texturized soybean protein, no horse meat, 6% egg albumin, 0.9% Human salt mix, 60 ml vitamin mix, 500 ml plasma
6. Texturized soybean protein, no horse meat, 1000 ml of a 1:1 dilution of hydroliquid, 500 ml plasma

Results

Group II Diets.-- Substitution of plasma for the water or the addition of egg albumin in the water of dilution produced slightly larger larvae (Table 1, diet 2) than did the Standard diet (Table 1, diet 1). However, they were not any larger than some larvae from the Standard diet in other groups (Table 1, Group III, diets 1 & 2; Group V, diet 1). Flies from diet 3 were the least responsive in the flight test (Table 2) and had one of the higher mortalities in response to stress (Table 3). Flies from diet 6 were most responsive in the flight test (Table 2) and had one of the lower stress mortalities (Table 3).

Group III Diets. --Adjustment of the pH to 7.0 did not make any consistent difference in larval weights, flight response, or stress response (Tables 1, 2, 3). Addition of lysine and phenylalanine to egg albumin (diets 5, 6) to correct a theoretical deficiency of these amino acids (based on amino acid analysis of the carcass of larvae which had developed on sheep) did not produce any change in larval weight (Table 1) or flight response (Table 2) but flies from this diet at pH 7.0 did have a higher stress mortality (Table 3).

Group IV Diets. --This group of diets was expected to be the most significant of those tested because the media was adjusted to pH 7.0, the concentrations of vitamins and minerals were adjusted to a more nearly correct amount than those in Group I and some modifications in the techniques to decrease waste and increase the availability of food had been made. Examination of the larval weights, flight tendency and stress response does not show any obvious differences between the Standard and the test diets.

Group V Diets. --These diets were to test the quality of different proteins. The larger larval weights in this group are attributed to the reduced number of larvae per container and the addition of plasma to make the diet more liquid during the last 24 hours of larval development. Larvae from diets 1, 2, and 3 are comparable. Diet 4 with 12% cottonseed flour (CSF) did not support growth beyond 24 hours, reduction to 6% CSF at the end of 24 hours improved growth somewhat and a further reduction to 3% CSF at the end of 48 hours permitted larvae in replicate 3 to mature. The CSF may have some value in diets at 3%. In diets 5 & 6, the texturized soybean protein was substituted for the meat and constituted approximately 50% of the diet; no development occurred on these diets.

The data were examined by plotting flight response against larval weight (Fig. 1). The data are from 18 different diets which might explain the scatter of the points. However, the distribution of the points suggest that the larger flies do have a greater tendency to fly. The use of a single diet and refinement of the flight response test should reduce the variation and confirm this observation.

The same variations from the 18 different diets are present in a plot of the stress mortality vs larval weight. This plot (Fig. 2) shows rather clearly that the mortality is less in flies from the larger larvae.

The survival value of the larger flies is further shown in a comparison of the dry weights of the survivors and dead from the stress test. At the end of the stress treatment period, the survivors were killed by crushing and then both survivors and dead were dried to a constant weight at 36° C for 24 hours and then weighed. It was found in 23 out of 29 comparisons that the survivors weigh more than those killed by the treatment (Table 4).

The wet weight of the survivors in Groups IV and V were determined (Table 5). It is seen that the survivors average about 25 mg or more regardless of the diet or replicate.

The conditions in a wound were simulated by the apparatus in Fig. 3. It provides for a continuous flow of plasma over the larvae to remove the waste products and supply fresh nutrients. Two types of experiments were done with this apparatus. In one, the plasma was made to flow over horsemeat; in another, the plasma flowed through cotton linters. Two groups of larvae from the horsemeat and plasma averaged 84.8 mg in the first group and 90 mg in the second group with the largest larva weighing 95 mg. Those from cotton linters only plus plasma weighed 50 mg.

Summary

1. Adult flies that have been killed by freezing or other methods will turn blue when exposed to temperatures near 36°C.
2. Wild flies reared on an animal and forced to pupate prematurely cannot be distinguished from media-reared flies.
3. Fresh meat diets and fresh Hydro diets have a pH of 6.0. Diet 24 hours old and "spent" Hydro have a pH of about 7.0. Based on larval weights, adjustment to pH 7.0 of the meat diets did not seem to make any difference in their growth (Group III diets).
4. The addition of protein to the diet was the only change which seemed to affect larval size. The effect of the variables of vitamins, minerals, pH, and even the type of protein appear to be obscured by the limitations imposed by the accumulation of waste products and/or the availability of food (especially during the last 24 hours).
5. Flies from large larvae appear to have the highest response in the flight tendency test (Table 2, Fig. 1) and this may indicate that the larger flies have a greater "biological vigor".
6. The flies from the larger larvae have the lowest mortality in the stress test. Weights of survivors and dead support the idea that the larger flies are the ones that survive the exposure to heat.
7. The findings in 5 and 6 above and Figs. 1 and 2 suggest that flies from larvae that weigh 68 mg or more have a higher flight response, less stress mortality, and probably live longer in nature than those weighing less.

1. Optimum age of the fly for testing.

2. Time interval of emergence of adults.

3. Pre-treatment.

a. Amount of CO₂ anesthesia and recovery time.

b. Interval between receiving food and water and the treatment.

Miscellaneous

1. One comparison of wild flies reared on cattle with those reared in the laboratory seemed to show a different type of activity and movement. Those from the lab seemed hyperactive with movements that were jerky and excessive. There seemed to be less waste movement in the wild flies. Other observations have not confirmed this but additional observations are suggested; if the type of movement in the lab flies is confirmed, it may indicate a deficiency of thiamine and/or niacin.
2. The question of the toxicity of formaldehyde has been raised on several occasions. If it is toxic, it may be due to its formic acid content. This might be checked and if formic acid is toxic, then the formaldehyde should be stored over calcium chips.
3. It was noted that benzene is used as a killing agent in the traps. Benzene is very toxic to humans and should be used with extreme caution. It is suggested that ethyl acetate be tried as substitute.

Conclusions

The original goal of producing a wild type of fly with a blue color and robust appearance was not accomplished, but the more general goal of determining if some nutritional changes in the rearing might produce a better quality fly was realized. The results of the studies indicated (1) that the waste products from the larvae probably limits their size, (2) that the lower limit on the size of acceptable larvae should be 65-68 mg instead of the present 60 mg, and (3) that larval size and the mortality resulting from exposure of adult flies to heat stress could give laboratory data that would have a direct relationship to survival in the field and consequently the performance of the released flies in the eradication program.

Recommendations for Additional Studies

- I. Stress Mortality.--The lower survival, under heat stress, of larvae that weigh less than 65 mg (Fig. 2) suggests that low survival of the released flies may have been a factor in the 1972 outbreak of screwworms in Texas.

The stress test appears to make possible the evaluation of the expected performance of the released "mass-reared" flies in the eradication program. It is suggested that first priority be given to refining and standardizing this test and relating the survival under laboratory conditions to survival in nature.

The following points of the test need to be standardized:

1. Optimum age of the fly for testing.
2. Time interval of emergence of adults.
3. Pre-treatment.
 - a. Amount of CO₂ anesthesia and recover time.
 - b. Interval between receiving food and water and the treatment.

4. Exposure temperature.

5. Length of exposure.

Relationship of survival in the lab test to that in nature might be accomplished in 2 ways: (1) Determine the survival of wild or animal-reared flies and use this data as an optimum or goal; (2) produce larvae that vary in size and determine the response of the resulting flies to laboratory stress and compare with the survival of similar flies in a standardized field cage test.

II. Nutritional Experiments. --The normal sized larvae which developed in the apparatus with horsemeat and continuously flowing plasma suggests that larval waste products limit the size of the larvae and also limits the effects of nutritional changes in the diet.

It is suggested that an effort be made to perfect an apparatus similar to that in Fig. 3 for use as a research tool. Dependable regulation of temperature and liquid flow (perhaps intermittent) through the larval medium would allow a more realistic appraisal of nutritional changes in the diet. For example, it was possible to produce 90 mg larvae with horsemeat and flowing plasma. What is the maximum size larvae that would be produced with a flow of Hydroponic fluid through the cotton linters? Such a device should be able to evaluate the quantity and quality of the ingredients in the Hydroponic diet and determine the most economical diet that would produce the best larvae because the waste products would be removed as a limiting factor. Use of such a flow-through technique would probably also reduce rearing costs by eliminating the need for removal of spent diet.

Efforts should also be made to determine what is in the waste that limits larval growth. Such information could make it possible to neutralize the wastes or remove the toxic component and reclaim the unused nutrients in the spent Hydro liquid.

Acknowledgements. --Many individuals contributed much to this research, both in actual assistance in the work and in discussions of the varied aspects of the screwworm program. Dr. Bushland, Dr. Gingrich, Jack Graham, and Frank Dutley were especially helpful in acquainting me with the screwworm program and providing basic information on the various aspects of rearing screwworms. Dr. Bushland, in addition, provided the necessary materials and facilities for the research. Thelma Jester was also helpful in securing materials and assistance. I greatly appreciate the willingness of Dr. M. M. Crystal to permit the free use of his laboratory space and equipment.

The services of Bob Handke are much appreciated. Bob was dependable in carrying out instructions, performed his duties with a minimum of instruction, anticipated the needs of the program, showed ingenuity in adapting laboratory apparatus to different needs, and he willingly worked additional hours outside the normal work schedule. I believe he is an excellent technician and should be encouraged to further increase his technical and formal training.

Abbreviations: EA-egg albumin; DB-dried blood; VM-Vanderant vitamin mix; AA-amino acids; HB-human blood salt; WS-Wesson salt; Hs-Huse salt; T-Mix-Tax-Max strain of flies; Ly-lysine; Ph-phenylalanine.

Table 1. Average weight^{a/} of larvae from experimental diets for the screwworm.

Diet	Replicate			Average
	1	2	3	
<u>Group II</u>				
Standard	1	65.8	69.6	67.7
1500 plasma	2	70.4	75.4	72.9
6% EA + AA	3	72.0	70.0	71.0
6% EA + AA, WS, VM	4	68.7	63.9	66.3
6% EA	5	77.9	74.6	76.3
6% EA, WS, 5% DB, VM	6	56.0	58.1	57.1
<u>Group III</u>				
Standard, pH Na	1	72.5	70.7	69.7
Standard, pH 7.0	2	71.8	71.1	70.6
Whole blood, pH Na	3	64.0	40.3	-
3% EA, pH 7.0	4	72.2	69.3	69.4
3% EA+Ly, Ph, pH 7.0	5	68.9	68.6	68.1
3% EA+Ly, Ph, pH Na	6	71.4	74.0	69.4
<u>Group IV</u>				
Standard	1	66.1	72.0	67.9
0.9% WS	2	58.2	65.2	63.1
0.9% HB	3	59.5	64.7	62.0
0.9% Ho	4	60.4	68.8	64.8
VM	5	65.0	63.5	67.1
3% EA, VM, 0.9% HB	6	60.6	60.1	60.1
<u>Group V</u>				
Standard	1	74.7	77.6	75.7
6% EA	2	74.4	77.5	76.5
6% Whole egg	3	81.7	70.6	72.6
12% cottonseed flour	4	-	-	62.5
Textured soybean protein, 6% EA	5	-	-	
Tex. soybean protein, 50% Hydro.	6	-	-	

Miscellaneous

Sheep lysine; Ph=phenylalanine 95.0 (pupae-78 mg)

Larvae from Apparatus with Plasma Exchange

Test 1 - 68 mg vs 69.7 (larvae)(regular): 53.7 (exchange) vs 50.8 (reg.)-pupae

Test 2 - 51.5 (exchange) vs 53.5 (reg.)-pupae

Test 3 - Meat 84.8 mg Test 4 - Meat 90 mg- largest 95 mg

Test 5 - Cotton linters 50 mg

^{a/} All weights in mg.

Abbreviations: EA=egg albumin; DB=dried blood; VM=Vanderzant vitamin mix; AA=amino acids; HB=Human blood salt; WS=Wesson salt; Ho=House salt; TxMx=Tex-Mex strain of flies; Ly=lysine; Ph=phenylalanine.

Table 2. Flight tendency of 5-day-old male screwworm flies; 3 replicates of 20 males each unless indicated otherwise. (Percent responding)

	Diet	Replicate			Average
		1	2	3	
<u>Group II</u>					
Standard	1	45		33	39
1500 plasma	2	45		32	39
6% EA + AA	3	40		24	32
6% EA + AA, WS, VM	4	38		32	36
6% EA	5	37		45	41
6% EA, WS, 5% DB, VM	6	53		48	51
Plant		20	33		27
Sheep		35			35
<u>Group III</u>					
Standard, pH Na	1	47	23	30	33
Standard, pH 7.0	2	55	23	17	32
Whole blood, pH Na	3	23	-	-	-
3% EA, pH 7.0	4	51	44	23	39
3% EA+Ly, Ph, pH 7.0	5	42	42	45	43
3% EA+Ly, Ph, pH Na	6	43	48	33	41
Plant		43	27	-	35
<u>Group IV</u>					
Standard	1	20	48.4	38.4	35.6
0.9% WS	2	15	41.8	47.1	34.6
0.9% HB	3	13.3	41.8	46.7	34.1
0.9% Ho	4	30	41.8	-	35.9
VM	5	18.3	36.7	46.7	33.9
3% EA, VM, 0.9% HB	6	25	38.4	-	31.7
<u>Group V</u>					
Standard	1	22	48	36	35
6% EA	2	23	27	34	24
6% Whole egg	3	20	11	34	22
12% cottonseed flour	4				
Tex. soybean protein, 6% EA	5				
Tex. soybean protein, 50% Hydro.	6				

Abbreviations: EA=egg albumin; DB=dried blood; VM=Vanderzant vitamin mix; AA=amino acids; HB=Human blood salt; WS=Wesson Salt; Ho= House salt; Ly=lysine; Ph=phenylalanine.

Table 3. Mortality of 5-day-old male screwworm flies after 16 hours exposure (without food or water) to 36°C. 20 males per replicate unless indicated otherwise. (Percent mortality)

Diet	Replicate			Average
	1	2	3	
<u>Group II</u>				
Standard	1	90	35	62.5
1500 plasma	2	85	100	92.5
6% EA + AA	3	75	100	87.5
6% EA+AA, WS, VM	4	60	90	85
6% EA	5	30	89	59.5
6% EA, WS, 5% DB, VM	6	55	70	62.5
Plant		100		100
Sheep		40	55	40
<u>Group III</u>				
Standard, pH Na	1	0	100	80
Standard, pH 7.0	2	15	70	72.5
Whole blood, pH Na	3	75	-	75
3% EA, pH 7.0	4	75	40	66.7
3% EA+Ly, Ph, pH 7.0	5	90	95	93.3
3% EA+Ly, Ph, pH Na		65	65	68.3
Plant		100	-	95
<u>Group IV</u>				
Standard	1	90	60	83.3
0.9% WS	2	95	95	95
0.9% HB	3	60	85	80
0.9% Ho	4	100	100	100
VM	5	95	100	97
3% EA, VM, 0.9% HB	6	80	85	82.5
Plant-TxMx		65	-	52.5
<u>Group V</u>				
Standard	1	50	60	65
6% EA	2	45	30	50
6% Whole egg	3	20	60	48
12% cottonseed flour	4		65	65
Tex. soybean protein, 6% EA	5			
Tex. soybean protein, 50% Hydro.	6			
Plant-TxMx		40	65	56.6

Abbreviations: EA=egg albumin; DB=dried blood; VM=Vanderzant vitamin mix; AA=amino acids; HB=Human blood salt; WS=Wesson salt; Ho=House salt; Ly=lysine; Ph=phenylalanine.

Table 4. Dry weights of flies that survived and died from heat stress.
(Weights in mg)

Diet	Replicate							
	1		2		3		Average	
	surv.	dead	surv.	dead	surv.	dead	surv.	dead
<u>Group III</u>								
1 Standard, pH Na					9.5	9.1		
2 Standard, pH 7.0					10.7	8.8		
3 Whole blood, pH Na								
4 3% EA, pH 7.0					10.2	10.0		
5 3% EA+Ly, Ph, pH 7.0					10.3	8.9		
6 3% EA+Ly, Ph, pH Na					9.0	9.3		
Average					9.9	9.2		
<u>Group IV</u>								
1 Standard	9.9	9.1		9.9	9.9	9.4	9.9	9.3
2 0.9% WS	9.0	8.2	8.7	8.3	9.0	8.7	8.9	8.4
3 0.9% HB	8.5	8.0	8.6	8.8	8.0	8.5	8.4	8.4
4 0.9% Ho	-	7.8		9.3		9.0		
5 VM	9.7	8.7		9.0		10.0	9.7	8.7
6 3% EA, VM, 0.9% HB	8.7	8.9		9.1			8.7	8.9
Plant(TxMx)	8.7	8.5	9.3	8.4				
Average	9.1	8.4*	8.7	8.5*	9.0	8.9*	9.1	8.7
<u>Group V</u>								
1 Standard	11.2	10.1	13.1	11.1	11.2	10.9	11.8	10.7
2 6% EA	11.4	10.8		10.4	11.1	11.3	11.3	11.1
3 6% whole egg	12.2	11.4		9.6	11.5	10.8	11.9	11.1
4 12% cottonseed flour					9.7	9.0		
5 Tex. soybean pro- tein, 6% EA								
6 Tex. soybean pro- tein, 50% Hydro.								
TxMx	9.0	8.2	9.5	8.1	9.6	8.0	9.4	8.1
Average	11.0	10.1	13.1	9.8	10.6	10.0	11.1	10.3

Abbreviations: EA=egg albumin; DB= dried blood; VM= Vanderzant vitamin mix;
AA= amino acids; HB=Human blood salt; WS=Wesson salt; Ho=House salt;
Ly=lysine; Ph=phenylalanine; TxMx=Tex-Mex strain of flies.

* Averages do not include values where 100% mortality occurred.

Table 5. The weight of male flies that survived heat stress. (Weights in mg)

Diet	Replicate			Average
	1	2	3	
<u>Group IV</u>				
1 Standard	29.1	-	22.1	25.6
2 0.9% WS	26.9	25.8	27.6	26.8
3 0.9% HB	23.1	24.8	22.7	23.5
4 0.9% Ho	-	-	-	-
5 VM	25.1			25.1
6 3% EA, VM, 0.9% HB	25.6	25.2		25.4
Plant-TxMx	23.2	25.1		24.1
Average	25.5	25.2	24.1	25.1
<u>Group V</u>				
1 Standard	33.5	37.6		35.6
2 6% EA	33.9	29.9		31.9
3 6% whole egg	35.2	30.6		32.9
4 12% cottonseed flour				
5 Tex. soybean protein, 6% EA				
6 Tex. soybean protein, 50% Hydro.				
Plant TxMx	25.0	26.8		
Average	31.9	31.2		33.5

Abbreviations: EA=egg albumin; DB=dried blood; VM=Vanderzant vitamin mix; AA=amino acids; HB=Human blood salt; WS=Wesson salt; TxMx=Tex-Mex strain of flies; Ho=House salt.

Table 6. Comparison of larval weight, stress mortality, and flight response of flies from four different sources.

Source	No. of replicate	Avg. larval weight (mg)	Stress % mortality	Flight % response
Sheep - Florida strain	2	95	53	35
Horsemeat standard-Florida strain	12	70.2	70	62
Hydroponic - APHIS	3	61 *	97	31
Hydroponic - TxMx	5	61 *	55	-

* Average weight of flies being produced by the Rearing Plant at the time the tests were conducted.

FIG. 1 FLIGHT RESPONSE OF ADULT FLIES IN RELATION TO LARVAL WEIGHT

80.

Group II •

Group III x

Group IV o

Group V ▲

75

Larval weight

70

65

60

% Response

56

20

30

40

50

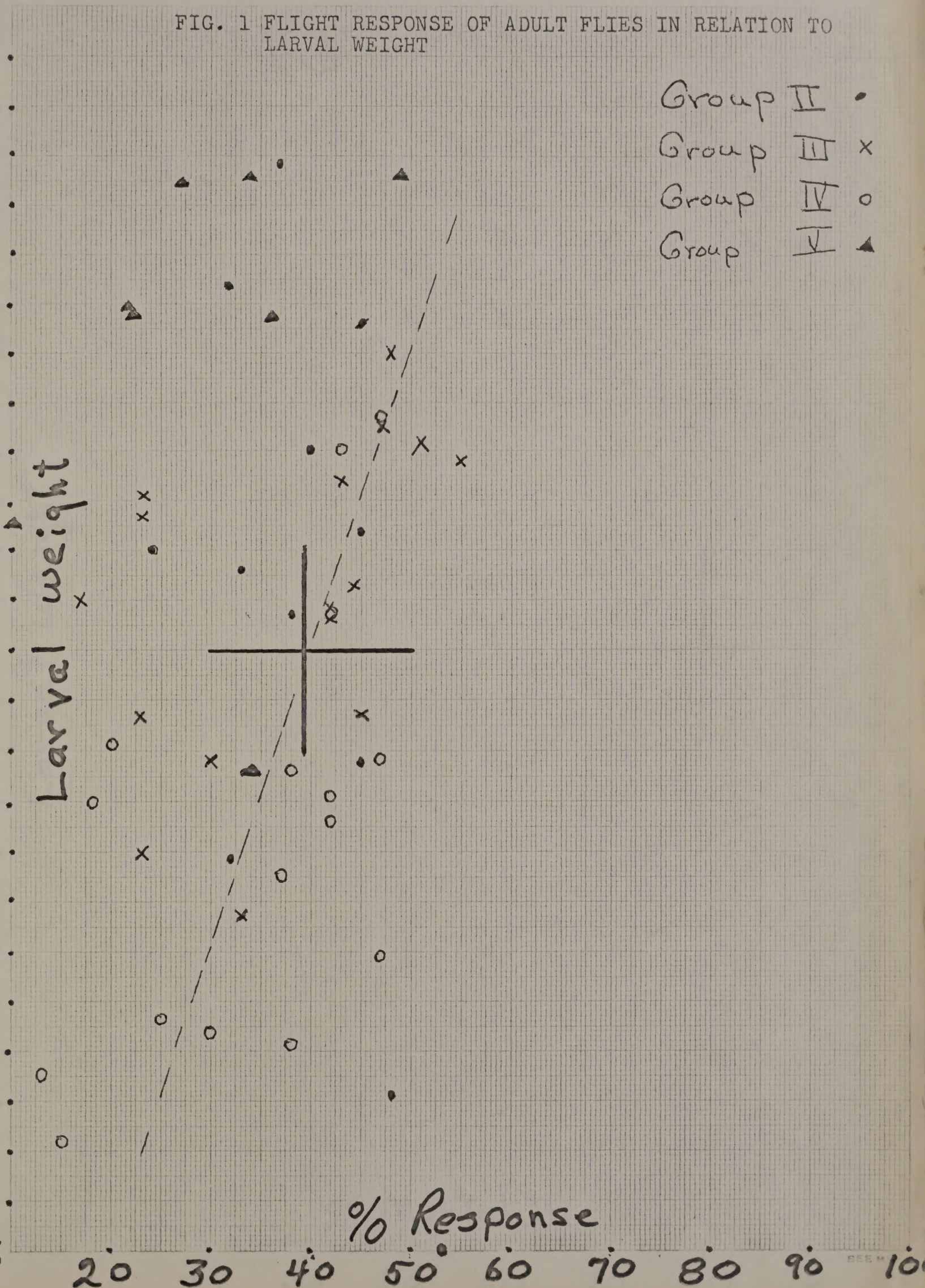
60

70

80

90

100



80

FIG. 2 THE RELATIONSHIP BETWEEN LARVAL WEIGHTS AND MORTALITY OF ADULT FLIES DUE TO EXPOSURE TO 36° , WITHOUT FOOD OR WATER FOR 16 HOURS

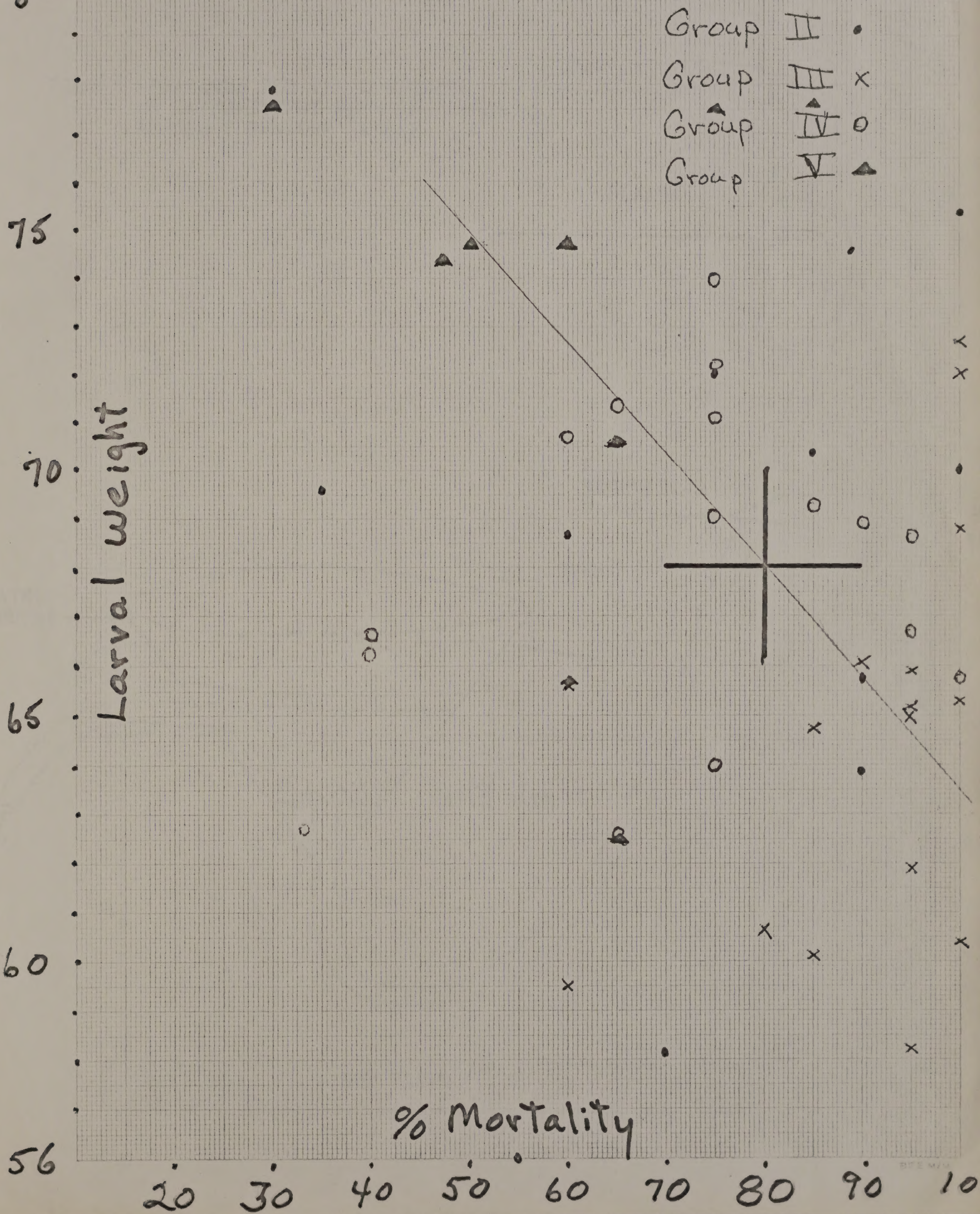
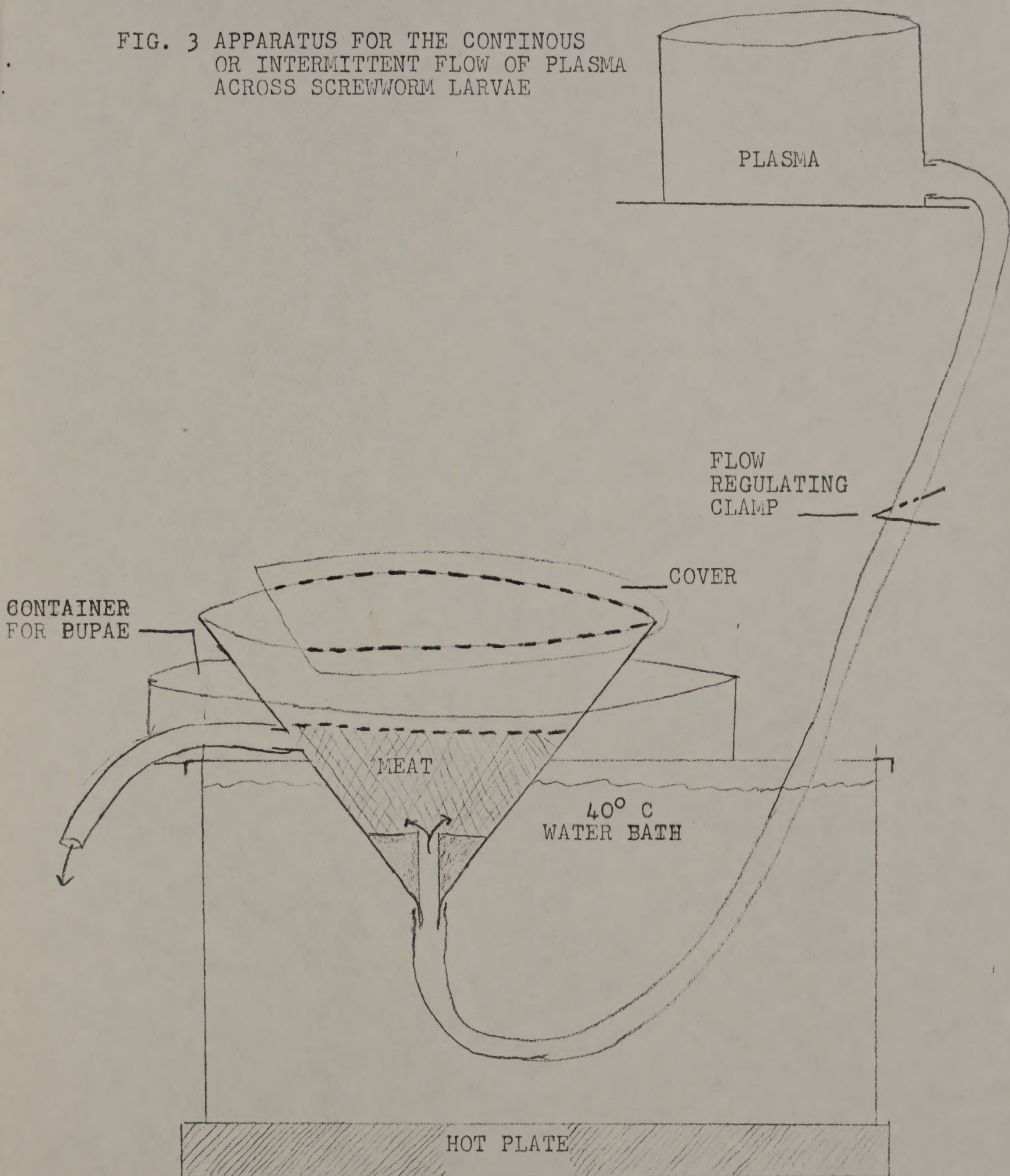
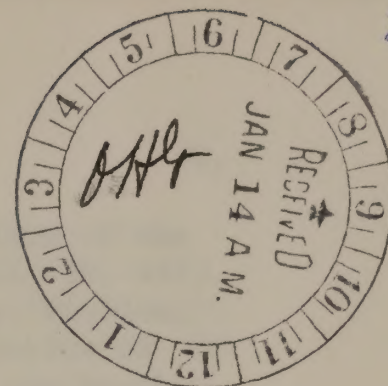


FIG. 3 APPARATUS FOR THE CONTINUOUS
OR INTERMITTENT FLOW OF PLASMA
ACROSS SCREWORM LARVAE



UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
Southern Region
Georgia -South Carolina Area
Southeastern Cotton Insects Investigations
P. O. Box 271, Florence, S. C. 29501



January 9, 1974

Subject: Report of Nutritional Research Conducted on Screwworm,
Oct. 29, 1973 to Dec. 12, 1973, at Mission, Texas

To:	W. M. Bruce, Tifton, Ga.	Lyman Henderson, Beltsville, Md.
	R. C. Bushland, Mission, Tex.	Chris Hoffman, Mission, Tex.
	E. Corristan, Mission, Tex.	C. H. Hoffman, Beltsville, Md.
	M. M. Crystal, Mission, Tex.	W. Klassen, Beltsville, Md.
	H C Cox, New Orleans, La.	E. F. Knipling, Beltsville, Md.
	R. E. Gingrich, Kerrville, Tex.	M. E. Meadows, Mission, Tex.
	Jack Graham, Mission, Tex.	E. A. Taylor, Weslaco, Tex.

Through: H. M. Taft, Research Leader

The subject report is the result of research conducted during a temporary assignment to the Screwworm Research Laboratory, Mission, Texas.

The original goal of producing a wild type of fly with a blue color and robust appearance was not accomplished, but the more general goal of determining if some nutritional changes in the rearing might produce a better quality fly was realized. The results of the studies indicated (1) that the waste products from the larvae probably limits their size, (2) that the lower limits on the size of acceptable larvae should be 65-68 mg instead of the present 60 mg, and (3) that larval size and the mortality resulting from exposure of adult flies to heat stress could give laboratory data that would have a direct relationship to survival in the field and consequently the performance of the released flies in the eradication program.

Some additional experiments will be conducted in January and February 1974 to complete portions of the studies.

R. F. Moore, Jr.

R. F. Moore, Jr.
Research Entomologist

REPORT OF NUTRITIONAL STUDIES ON THE SCREWORM
CONDUCTED AT THE SCREWORM RESEARCH LABORATORY

Mission, Texas

October 29 - December 12, 1973

by

R. F. Moore, Jr. Research Entomologist
Southeastern Cotton Insects Investigations
Florence, S. C. 29501

Dr. R. E. Gingrich and I were requested to review the nutrition of the screwworm in a memorandum from Dr. Waldemar Klassen dated Feb. 26, 1973. Dr. Gingrich and I visited the Screwworm Research Laboratory, Mission, Texas in early April 1973 and plans were made to conduct a series of experiments, to begin in the fall and lasting approximately one month. The experiments were to be designed to produce, from a laboratory diet, a fly with the "blue" color and "broad" thorax of the wild or animal-reared fly.

To assist in the experimental design, information on the composition of beef blood, amino acid content of blood and muscle, and an amino acid analysis of screwworm larvae and adults was requested. Dr. Klassen assisted in routing this request to the proper individuals so that the data were available for the experiments.

I arrived in McAllen, Texas on Oct. 28, 1973 and reported to the Screwworm Research Laboratory on Monday, Oct. 29. Dr. Gingrich was also there and after consultation with Dr. R. C. Bushland and Jack Graham, a series of experiments was planned.

The available data indicated that a "wild" type of fly had not been produced, even on the best diets of horsemeat, blood and plasma. Thus, it seemed advisable to attempt to make changes in the "best" diet to determine if it could be modified to produce a wild type fly. Examination of the experiments with horsemeat: blood: plasma diets revealed that the blood or plasma was always diluted with water by a factor of 1/3 to 1/2.

The reasons for the limitation on color, robustness, and larval size of insects from laboratory diets are not known. They may be related to the differences between "living" and "dead" tissue; such differences would be subtle, perhaps complex and difficult to determine. It was felt that a deficiency in some major component of the media, such as protein, minerals or vitamins, should be eliminated as a first step. Therefore, protein as egg albumin, mineral mixtures, or a vitamin mixture were added to the 1000 ml of water and in addition, plasma was substituted for the 1000 ml of water to determine if dilution of one or all of these components determined the type of fly produced from the diets. Availability of food, the accumulation of wastes, and pH were also recognized as possible limitations on the type of fly and some experiments were designed to test these factors.

The original intention was to observe the incidence of blue flies and make some measurements of the thorax as an indication of the success of the modifications of the diet. It was soon apparent that there was considerable variation in the color and appearance in both lab and wild flies and that valid objective measurements of the differences would be difficult. Discussions with individuals associated with the Screwworm Project raised several questions about the color of wild flies and the factor(s) that make the wild fly appear more "broad shouldered" or robust. The incidence of "green" screwworm flies in nature, the size variation and the effect of premature pupation (forced by death of the host) on color and robustness of the flies are other unknowns.

Some individuals felt that there was a color change in the flies after death. This was tested by freezing some laboratory-reared flies, then holding some at room temperature, heating some at 37°C for several hours, and putting others in the sun. Those heated at 37° or exposed to the sun changed to the blue color of the wild fly. Flies which are caught in traps might be exposed to such conditions either in the trap or in the vehicle in which they are transported. Thus, an unknown number of green wild flies may change to blue in the trapping and handling process. Captured laboratory flies might also turn blue and be incorrectly identified as wild.

Dr. Bill Hightower had, in the past, made measurements of the width and length of the thorax of wild and laboratory-reared flies. The ratio of length/width from these data was found to be 1.02 ± 0.02 for 25 wild flies and 1.05 ± 0.02 for 25 lab flies. This is not statistically significant by the t test. Efforts to find specific characteristics to measure the "robust" characteristic were unsuccessful.

The robust characteristic was thought to be present, even in small wild flies which result from premature pupation. This was discussed with Mr. Calvin Jones and at his suggestion a field trip was made to the King Ranch at Encino, Texas. Larvae in the ears of cattle were obtained; a group of large larvae were forced to pupate without completing their feeding and produced pupae weighing 49 mg. This corresponds to a larval weight of wild flies of approximately 70 mg but would be slightly larger than the usual laboratory-reared larvae. A second group of larvae was about 24 hours old; these were forced into the horse meat diet where development was completed and pupae weighing 53.9 mg were produced. Mr. Jack Graham examined the adult flies from these larvae and compared them with flies that had been reared on sheep, on hydroponic diets, and horse meat diets. Only those from sheep had the appearance of wild flies. Thus, flies which had developed from larvae taken from cattle and forced to pupate prematurely or which completed a substantial portion of their larval development on laboratory diets could not be distinguished from flies reared wholly on laboratory diets. In other words, both blue and green flies were present and they did not have the robust appearance of wild flies. It was concluded that the blue color and broad thorax or robust appearance were difficult to define and were inadequate for evaluation of differences in flies from the experimental diets.

Since the original characteristics were lacking in objectivity, the following methods were selected for evaluation of the flies from the experimental diets: (1) Average larval weight as determined by the weight of 10 larvae which were selected just prior to pupation; (2) Tendency to fly-- this was a modification of a published method for determining the tendency of an insect to fly. Modification of the method, equipment, and initial testing had been done by Dr. M. M. Crystal. This method was applied to the males from each diet in the expectation that flies from the best diets might have a greater tendency to fly; (3) Stress survival-- Jack Graham had done some experiments with the survival of adult flies at 39°C. It was reasoned that survival under this type of stress would be a desirable characteristic and such flies should be found in the best

diets. In the test, 20 male flies that were 5 days old were exposed to 36°C, without food or water for 16 hours and the percent mortality recorded; (4) Categorizing by physical characteristics-- 3 technicians, experienced in the classification of wild and laboratory-reared flies, classified 20 male flies from each diet. A successful diet should show a larger percentage of flies appearing like the wild type.

Examination of the results of the evaluation tests of flies from the Group III diets suggested the possibility that the size of the flies might be a factor in survival under the stress of exposure, without food or water, to a temperature of 36° for 16 hours. Therefore, some measurements of the wet weights of survivors were made immediately at the end of the test. Measurements of the dry weights of the survivors and dead flies were made after drying to a constant weight at 36°C.

Five groups of experimental diets were set up. Each group contained a Standard diet which contained 4 ml formaldehyde, 1000 ml water, 500 ml blood plasma, and macerated horse meat (approximately 1500 gm) for a proper consistency. Unless otherwise indicated, the changes in the diets were to modify the 1000 ml of water by adding egg albumin, minerals, vitamins or by substitution of plasma for the water.

The diets were made up on Friday afternoon and one replicate of each diet was started on Sat., Sun., and Mon. The larvae pupated in 4 days and emerged as adults in 15 days. The adults were tested when 5 days old.

The diets were:

Group I*

1. Standard
2. 0.1% Wesson salts
3. 0.1% Human blood salt mix
4. 0.1% House salt mix
5. 20 ml of Vanderzant vitamin mix

* Dr. Gingrich determined the osmotic pressure of the salts after the tests were begun and found that 0.9% was more nearly isotonic. It was also found that the vitamins were only 1/3 of that which might be required. Thus, flies from this group were used in an effort to standardize the evaluation procedures.

Group II

1. Standard
2. Undiluted plasma
3. 6% egg albumin + amino acids*
4. 6% egg albumin + amino acids, 0.1% Wesson salts, 20 ml vitamins
5. 6% egg albumin
6. 6% egg albumin + amino acids, 5% dried blood, 0.1% Wesson salts, 20 ml vitamin mix

* Selected amino acids were added to the egg albumin to make its amino acid composition more like that of bovine plasma.

Group III

1. Standard - pH not adjusted
2. Standard - pH 7.0
3. 1000 ml citrated whole blood, pH not adjusted
4. 3% egg albumin, pH 7.0
5. 3% egg albumin + Lysine and Phenylalanine*, pH 7.0
6. 3% egg albumin + Lysine and Phenylalanine*, pH not adjusted

* The "protein score" concept was used as the basis for modifying the amino acid composition of the egg albumin. The carcass analysis of larvae reared on sheep was used as the standard and lysine and phenylalanine were the most limiting amino acids.

Group IV - all diets were pH 7.0

1. Standard
2. 0.9% Wesson salt mix
3. 0.9% Human blood salt mix
4. 0.9% House salt mix
5. 60 ml Vanderzant vitamin mix
6. 3% egg albumin, 60 ml vitamin mix, 0.9% Human salt mix

Group V - all diets were pH 7.0

1. Standard
2. 6% egg albumin
3. 6% whole egg
4. 12% cottonseed flour
5. Texturized soybean protein, no horse meat, 6% egg albumin, 0.9% Human salt mix, 60 ml vitamin mix, 500 ml plasma
6. Texturized soybean protein, no horse meat, 1000 ml of a 1:1 dilution of hydroliquid, 500 ml plasma

Results

Group II Diets.-- Substitution of plasma for the water or the addition of egg albumin in the water of dilution produced slightly larger larvae (Table 1, diet 2) than did the Standard diet (Table 1, diet 1). However, they were not any larger than some larvae from the Standard diet in other groups (Table 1, Group III, diets 1 & 2; Group V, diet 1). Flies from diet 3 were the least responsive in the flight test (Table 2) and had one of the higher mortalities in response to stress (Table 3). Flies from diet 6 were most responsive in the flight test (Table 2) and had one of the lower stress mortalities (Table 3).

Group III Diets.--Adjustment of the pH to 7.0 did not make any consistent difference in larval weights, flight response, or stress response (Tables 1, 2, 3). Addition of lysine and phenylalanine to egg albumin (diets 5, 6) to correct a theoretical deficiency of these amino acids (based on amino acid analysis of the carcass of larvae which had developed on sheep) did not produce any change in larval weight (Table 1) or flight response (Table 2) but flies from this diet at pH 7.0 did have a higher stress mortality (Table 3).

Group IV Diets.--This group of diets was expected to be the most significant of those tested because the media was adjusted to pH 7.0, the concentrations of vitamins and minerals were adjusted to a more nearly correct amount than those in Group I and some modifications in the techniques to decrease waste and increase the availability of food had been made. Examination of the larval weights, flight tendency and stress response does not show any obvious differences between the Standard and the test diets.

Group V Diets.--These diets were to test the quality of different proteins. The larger larval weights in this group are attributed to the reduced number of larvae per container and the addition of plasma to make the diet more liquid during the last 24 hours of larval development. Larvae from diets 1, 2, and 3 are comparable. Diet 4 with 12% cottonseed flour (CSF) did not support growth beyond 24 hours, reduction to 6% CSF at the end of 24 hours improved growth somewhat and a further reduction to 3% CSF at the end of 48 hours permitted larvae in replicate 3 to mature. The CSF may have some value in diets at 3%. In diets 5 & 6, the texturized soybean protein was substituted for the meat and constituted approximately 50% of the diet; no development occurred on these diets.

The data were examined by plotting flight response against larval weight (Fig. 1). The data are from 18 different diets which might explain the scatter of the points. However, the distribution of the points suggest that the larger flies do have a greater tendency to fly. The use of a single diet and refinement of the flight response test should reduce the variation and confirm this observation.

The same variations from the 18 different diets are present in a plot of the stress mortality vs larval weight. This plot (Fig. 2) shows rather clearly that the mortality is less in flies from the larger larvae.

The survival value of the larger flies is further shown in a comparison of the dry weights of the survivors and dead from the stress test. At the end of the stress treatment period, the survivors were killed by crushing and then both survivors and dead were dried to a constant weight at 36° C for 24 hours and then weighed. It was found in 23 out of 29 comparisons that the survivors weigh more than those killed by the treatment (Table 4).

The wet weight of the survivors in Groups IV and V were determined (Table 5). It is seen that the survivors average about 25 mg or more regardless of the diet or replicate.

The conditions in a wound were simulated by the apparatus in Fig. 3. It provides for a continuous flow of plasma over the larvae to remove the waste products and supply fresh nutrients. Two types of experiments were done with this apparatus. In one, the plasma was made to flow over horsemeat; in another, the plasma flowed through cotton linters. Two groups of larvae from the horsemeat and plasma averaged 84.8 mg in the first group and 90 mg in the second group with the largest larva weighing 95 mg. Those from cotton linters only plus plasma weighed 50 mg.

Summary

1. Adult flies that have been killed by freezing or other methods will turn blue when exposed to temperatures near 36°C.
2. Wild flies reared on an animal and forced to pupate prematurely cannot be distinguished from media-reared flies.
3. Fresh meat diets and fresh Hydro diets have a pH of 6.0. Diet 24 hours old and "spent" Hydro have a pH of about 7.0. Based on larval weights, adjustment to pH 7.0 of the meat diets did not seem to make any difference in their growth (Group III diets).
4. The addition of protein to the diet was the only change which seemed to affect larval size. The effect of the variables of vitamins, minerals, pH, and even the type of protein appear to be obscured by the limitations imposed by the accumulation of waste products and/or the availability of food (especially during the last 24 hours).
5. Flies from large larvae appear to have the highest response in the flight tendency test (Table 2, Fig. 1) and this may indicate that the larger flies have a greater "biological vigor".
6. The flies from the larger larvae have the lowest mortality in the stress test. Weights of survivors and dead support the idea that the larger flies are the ones that survive the exposure to heat.
7. The findings in 5 and 6 above and Figs. 1 and 2 suggest that flies from larvae that weigh 68 mg or more have a higher flight response, less stress mortality, and probably live longer in nature than those weighing less.

1. Optimum age of the fly for testing.
2. Time interval of emergence of adults.
3. Pre-treatment.
 - a. Amount of CO₂ anesthesia and recover time.
 - b. Interval between receiving food and water and the treatment.

Miscellaneous

1. One comparison of wild flies reared on cattle with those reared in the laboratory seemed to show a different type of activity and movement. Those from the lab seemed hyperactive with movements that were jerky and excessive. There seemed to be less waste movement in the wild flies. Other observations have not confirmed this but additional observations are suggested; if the type of movement in the lab flies is confirmed, it may indicate a deficiency of thiamine and/or niacin.
2. The question of the toxicity of formaldehyde has been raised on several occasions. If it is toxic, it may be due to its formic acid content. This might be checked and if formic acid is toxic, then the formaldehyde should be stored over calcium chips.
3. It was noted that benzene is used as a killing agent in the traps. Benzene is very toxic to humans and should be used with extreme caution. It is suggested that ethyl acetate be tried as substitute.

Conclusions

The original goal of producing a wild type of fly with a blue color and robust appearance was not accomplished, but the more general goal of determining if some nutritional changes in the rearing might produce a better quality fly was realized. The results of the studies indicated (1) that the waste products from the larvae probably limits their size, (2) that the lower limit on the size of acceptable larvae should be 65-68 mg instead of the present 60 mg, and (3) that larval size and the mortality resulting from exposure of adult flies to heat stress could give laboratory data that would have a direct relationship to survival in the field and consequently the performance of the released flies in the eradication program.

Recommendations for Additional Studies

I. Stress Mortality.--The lower survival, under heat stress, of larvae that weigh less than 65 mg (Fig. 2) suggests that low survival of the released flies may have been a factor in the 1972 outbreak of screwworms in Texas.

The stress test appears to make possible the evaluation of the expected performance of the released "mass-reared" flies in the eradication program. It is suggested that first priority be given to refining and standardizing this test and relating the survival under laboratory conditions to survival in nature.

The following points of the test need to be standardized:

1. Optimum age of the fly for testing.
2. Time interval of emergence of adults.
3. Pre-treatment.
 - a. Amount of CO₂ anesthesia and recover time.
 - b. Interval between receiving food and water and the treatment.

4. Exposure temperature.
5. Length of exposure.

Relationship of survival in the lab test to that in nature might be accomplished in 2 ways: (1) Determine the survival of wild or animal-reared flies and use this data as an optimum or goal; (2) produce larvae that vary in size and determine the response of the resulting flies to laboratory stress and compare with the survival of similar flies in a standardized field cage test.

II. Nutritional Experiments. --The normal sized larvae which developed in the apparatus with horsemeat and continuously flowing plasma suggests that larval waste products limit the size of the larvae and also limits the effects of nutritional changes in the diet.

It is suggested that an effort be made to perfect an apparatus similar to that in Fig. 3 for use as a research tool. Dependable regulation of temperature and liquid flow (perhaps intermittent) through the larval medium would allow a more realistic appraisal of nutritional changes in the diet. For example, it was possible to produce 90 mg larvae with horsemeat and flowing plasma. What is the maximum size larvae that would be produced with a flow of Hydroponic fluid through the cotton linters? Such a device should be able to evaluate the quantity and quality of the ingredients in the Hydroponic diet and determine the most economical diet that would produce the best larvae because the waste products would be removed as a limiting factor. Use of such a flow-through technique would probably also reduce rearing costs by eliminating the need for removal of spent diet.

Efforts should also be made to determine what is in the waste that limits larval growth. Such information could make it possible to neutralize the wastes or remove the toxic component and reclaim the unused nutrients in the spent Hydro liquid.

Acknowledgements. --Many individuals contributed much to this research, both in actual assistance in the work and in discussions of the varied aspects of the screwworm program. Dr. Bushland, Dr. Gingrich, Jack Graham, and Frank Dutley were especially helpful in acquainting me with the screwworm program and providing basic information on the various aspects of rearing screwworms. Dr. Bushland, in addition, provided the necessary materials and facilities for the research. Thelma Jester was also helpful in securing materials and assistance. I greatly appreciate the willingness of Dr. M. M. Crystal to permit the free use of his laboratory space and equipment.

The services of Bob Handke are much appreciated. Bob was dependable in carrying out instructions, performed his duties with a minimum of instruction, anticipated the needs of the program, showed ingenuity in adapting laboratory apparatus to different needs, and he willingly worked additional hours outside the normal work schedule. I believe he is an excellent technician and should be encouraged to further increase his technical and formal training.

Table 1. Average weight^{a/} of larvae from experimental diets for the screwworm.

	Diet	Replicate			Average
		1	2	3	
<u>Group II</u>					
Standard	1	65.8		69.6	67.7
1500 plasma	2	70.4		75.4	72.9
6% EA + AA	3	72.0		70.0	71.0
6% EA + AA, WS, VM	4	68.7		63.9	66.3
6% EA	5	77.9		74.6	76.3
6% EA, WS, 5% DB, VM	6	56.0		58.1	57.1
<u>Group III</u>					
Standard, pH Na	1	72.5	70.7	65.8	69.7
Standard, pH 7.0	2	71.8	71.1	69.0	70.6
Whole blood, pH Na	3	64.0	40.3	-	-
3% EA, pH 7.0	4	72.2	69.3	66.7	69.4
3% EA+Ly, Ph, pH 7.0	5	68.9	68.6	66.7	68.1
3% EA+Ly, Ph, pH Na	6	71.4	74.0	62.7	69.4
<u>Group IV</u>					
Standard	1	66.1	72.0	65.6	67.9
0.9% WS	2	58.2	65.2	65.9	63.1
0.9% HB	3	59.5	64.7	61.9	62.0
0.9% Ho	4	60.4	68.8	65.3	64.8
VM	5	65.0	63.5	72.7	67.1
3% EA, VM, 0.9% HB	6	60.6	60.1	59.5	60.1
<u>Group V</u>					
Standard	1	74.7	77.6	74.8	75.7
6% EA	2	74.4	77.5	77.6	76.5
6% Whole egg	3	81.7	70.6	65.6	72.6
12% cottonseed flour	4	-	-	62.5	62.6
Textured soybean protein, 6% EA	5	-	-		
Tex. soybean protein, 50% Hydro.	6	-	-		

Miscellaneous

Sheep 95.0 (pupae-78 mg)

Larvae from Apparatus with Plasma Exchange

Test 1 - 68 mg vs 69.7 (larvae)(regular): 53.7 (exchange) vs 50.8 (reg.)-pupae

Test 2 - 51.5 (exchange) vs 53.5 (reg.)-pupae

Test 3 - Meat 84.8 mg Test 4 - Meat 90 mg- largest 95 mg

Test 5 - Cotton linters 50 mg

^{a/} All weights in mg.

Abbreviations: EA=egg albumin; DB=dried blood; VM=Vanderzant vitamin mix;

AA=amino acids; HB=Human blood salt; WS=Wesson salt; Ho=House salt;

TxMx= Tex-Mex strain of flies; Ly=lysine; Ph=phenylalanine.

Table 2. Flight tendency of 5-day-old male screwworm flies; 3 replicates of 20 males each unless indicated otherwise. (Percent responding)

Diet	Replicate			Average
	1	2	3	
Group II				
Standard	1	45	33	39
1500 plasma	2	45	32	39
6% EA + AA	3	40	24	32
6% EA + AA, WS, VM	4	38	32	36
6% EA	5	37	45	41
6% EA, WS, 5% DB, VM	6	53	48	51
Plant		20	33	27
Sheep		35		35
Group III				
Standard, pH Na	1	47	23	33
Standard, pH 7.0	2	55	23	32
Whole blood, pH Na	3	23	-	-
3% EA, pH 7.0	4	51	44	39
3% EA+Ly, Ph, pH 7.0	5	42	42	43
3% EA+Ly, Ph, pH Na	6	43	48	41
Plant		43	27	35
Group IV				
Standard	1	20	48.4	35.6
0.9% WS	2	15	41.8	34.6
0.9% HB	3	13.3	41.8	34.1
0.9% Ho	4	30	41.8	35.9
VM	5	18.3	36.7	33.9
3% EA, VM, 0.9% HB	6	25	38.4	31.7
Group V				
Standard	1	22	48	35
6% EA	2	23	27	24
6% Whole egg	3	20	11	22
12% cottonseed flour	4			
Tex. soybean protein,				
6% EA	5			
Tex. soybean protein,				
50% Hydro.	6			

Abbreviations: EA=egg albumin; DB=dried blood; VM=Vanderzant vitamin mix; AA=amino acids; HB=Human blood salt; WS=Wesson Salt; Ho= House salt; Ly=lysine; Ph=phenylalanine.

Table 3. Mortality of 5-day-old male screwworm flies after 16 hours exposure (without food or water) to 36°C. 20 males per replicate unless indicated otherwise. (Percent mortality)

	Diet	Replicate			Average
		1	2	3	
<u>Group II</u>					
Standard	1	90		35	62.5
1500 plasma	2	85		100	92.5
6% EA + AA	3	75		100	87.5
6% EA+AA, WS, VM	4	60		90	85
6% EA	5	30		89	59.5
6% EA, WS, 5% DB, VM	6	55		70	62.5
Plant		100			100
Sheep		40	55		40
<u>Group III</u>					
Standard, pH Na	1	0	60	100	80
Standard, pH 7.0	2	15	75	70	72.5
Whole blood, pH Na	3	75	-	-	75
3% EA, pH 7.0	4	75	85	40	66.7
3% EA+Ly, Ph, pH 7.0	5	90	95	95	93.3
3% EA+Ly, Ph, pH Na		65	75	65	68.3
Plant		100	90	-	95
<u>Group IV</u>					
Standard	1	90	100	60	83.3
0.9% WS	2	95	95	95	95
0.9% HB	3	60	85	95	80
0.9% Ho	4	100	100	100	100
VM	5	95	100	100	97
3% EA, VM, 0.9% HB	6	80	85	-	82.5
Plant-TxMx		65	40	-	52.5
<u>Group V</u>					
Standard	1	50	85	60	65
6% EA	2	45	75	30	50
6% Whole egg	3	20	65	60	48
12% cottonseed flour	4			65	65
Tex. soybean protein, 6% EA	5				
Tex. soybean protein, 50% Hydro.	6				
Plant-TxMx		40	65	65	56.6

Abbreviations: EA=egg albumin; DB=dried blood; VM=Vanderzant vitamin mix; AA=amino acids; HB=Human blood salt; WS=Wesson salt; Ho=House salt; Ly=lysine; Ph=phenylalanine.

Table 4. Dry weights of flies that survived and died from heat stress.
(Weights in mg)

Diet	Replicate							
	1		2		3		Average	
	surv.	dead	surv.	dead	surv.	dead	surv.	dead
Group III								
1 Standard, pH Na					9.5	9.1		
2 Standard, pH 7.0					10.7	8.8		
3 Whole blood, pH Na								
4 3% EA, pH 7.0					10.2	10.0		
5 3% EA+Ly, Ph, pH 7.0					10.3	8.9		
6 3% EA+Ly, Ph, pH Na					9.0	9.3		
Average					9.9	9.2		
Group IV								
1 Standard	9.9	9.1		9.9	9.9	9.4	9.9	9.3
2 0.9% WS	9.0	8.2	8.7	8.3	9.0	8.7	8.9	8.4
3 0.9% HB	8.5	8.0	8.6	8.8	8.0	8.5	8.4	8.4
4 0.9% Ho	-	7.8		9.3		9.0		
5 VM	9.7	8.7		9.0		10.0	9.7	8.7
6 3% EA, VM, 0.9% HB	8.7	8.9		9.1			8.7	8.9
Plant(TxMx)	8.7	8.5	9.3	8.4				
Average	9.1	8.4*	8.7	8.5*	9.0	8.9*	9.1	8.7
Group V								
1 Standard	11.2	10.1	13.1	11.1	11.2	10.9	11.8	10.7
2 6% EA	11.4	10.8		10.4	11.1	11.3	11.3	11.1
3 6% whole egg	12.2	11.4		9.6	11.5	10.8	11.9	11.1
4 12% cottonseed flour					9.7	9.0		
5 Tex. soybean pro- tein, 6% EA								
6 Tex. soybean pro- tein, 50% Hydro.								
TxMx	9.0	8.2	9.5	8.1	9.6	8.0	9.4	8.1
Average	11.0	10.1	13.1	9.8	10.6	10.0	11.1	10.3

Abbreviations: EA=egg albumin; DB= dried blood; VM= Vanderzant vitamin mix;
AA= amino acids; HB=Human blood salt; WS=Wesson salt; Ho=House salt;
Ly=lysine; Ph=phenylalanine; TxMx=Tex-Mex strain of flies.

* Averages do not include values where 100% mortality occurred.

Table 5. The weight of male flies that survived heat stress. (Weights in mg)

Diet	Replicate			Average
	1	2	3	
<u>Group IV</u>				
1 Standard	29.1	-	22.1	25.6
2 0.9% WS	26.9	25.8	27.6	26.8
3 0.9% HB	23.1	24.8	22.7	23.5
4 0.9% Ho	-	-	-	-
5 VM	25.1			25.1
6 3% EA, VM, 0.9% HB	25.6	25.2		25.4
Plant-TxMx	23.2	25.1		24.1
Average	25.5	25.2	24.1	25.1
<u>Group V</u>				
1 Standard	33.5	37.6		35.6
2 6% EA	33.9	29.9		31.9
3 6% whole egg	35.2	30.6		32.9
4 12% cottonseed flour				
5 Tex. soybean protein, 6% EA				
6 Tex. soybean protein, 50% Hydro.				
Plant TxMx	25.0	26.8		
Average	31.9	31.2		33.5

Abbreviations: EA=egg albumin; DB=dried blood; VM=Vanderzant vitamin mix; AA=amino acids; HB=Human blood salt; WS=Wesson salt; TxMx=Tex-Mex strain of flies; Ho=House salt.

Table 6. Comparison of larval weight, stress mortality, and flight response of flies from four different sources.

Source	No. of replicate	Avg. larval weight (mg)	Stress % mortality	Flight % response
Sheep - Florida strain	2	95	53	35
Horsemeat standard- Florida strain	12	70.2	70	62
Hydroponic - APHIS	3	61 *	97	31
Hydroponic - TxMx	5	61 *	55	-

* Average weight of flies being produced by the Rearing Plant at the time the tests were conducted.

FIG. 1 FLIGHT RESPONSE OF ADULT FLIES IN RELATION TO LARVAL WEIGHT

80.

Group II •
Group III x
Group IV o
Group V ▲

75

70

Larval weight

65

60

% Response

56

20

30

40

50

60

70

80

90

100

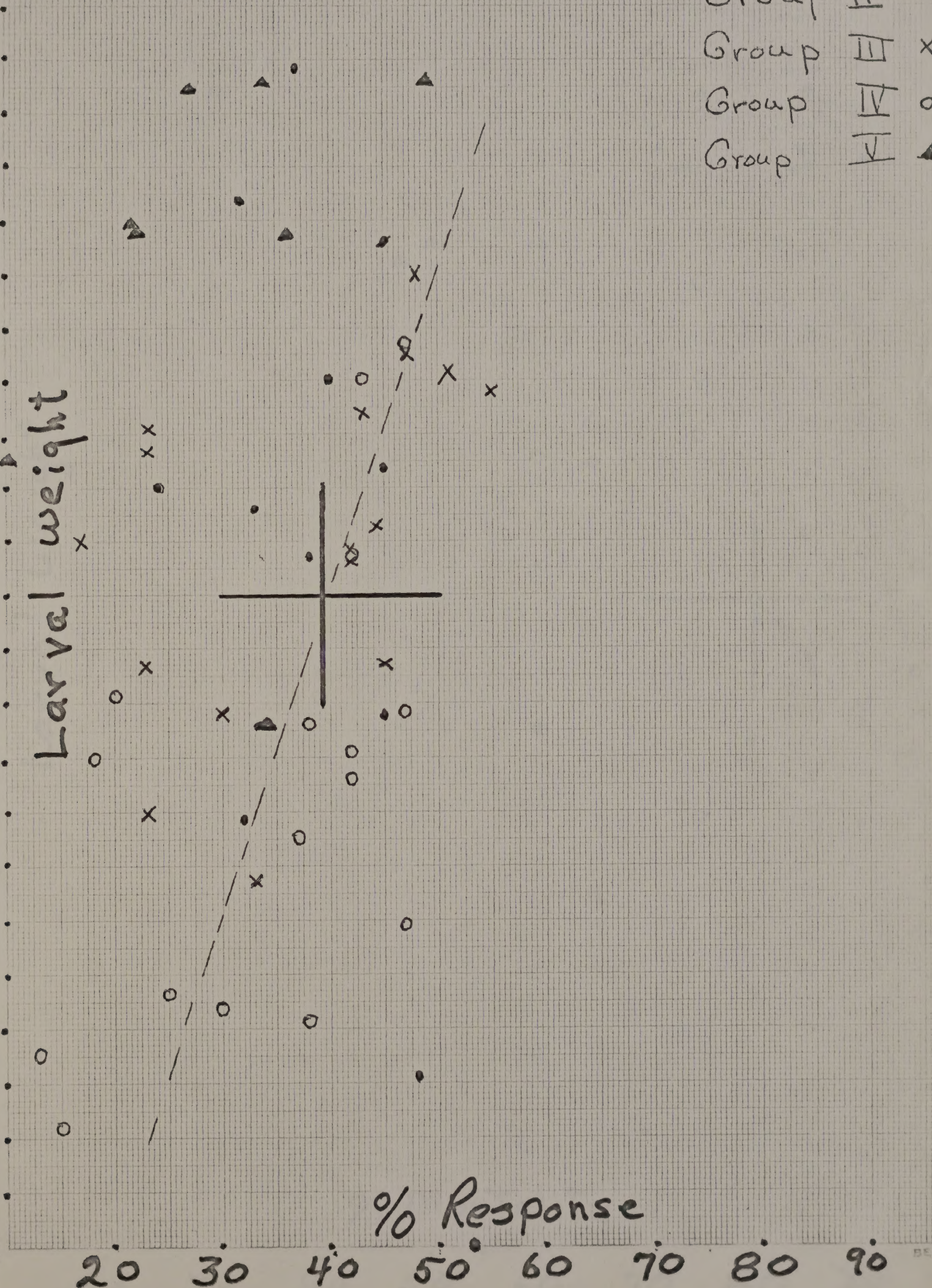


FIG. 2 THE RELATIONSHIP BETWEEN LARVAL WEIGHTS AND MORTALITY OF ADULT FLIES DUE TO EXPOSURE TO 36°, WITHOUT FOOD OR WATER FOR 16 HOURS

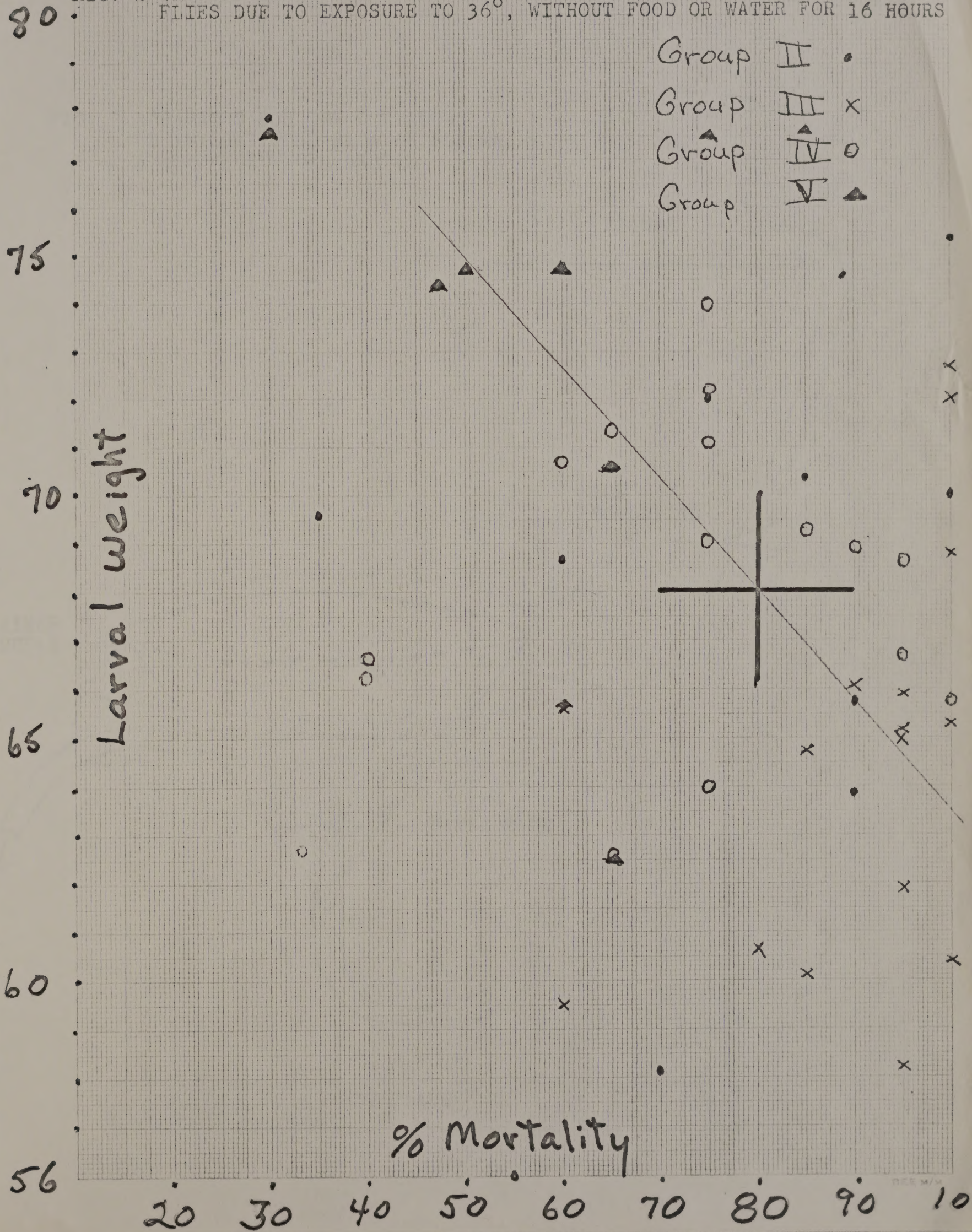
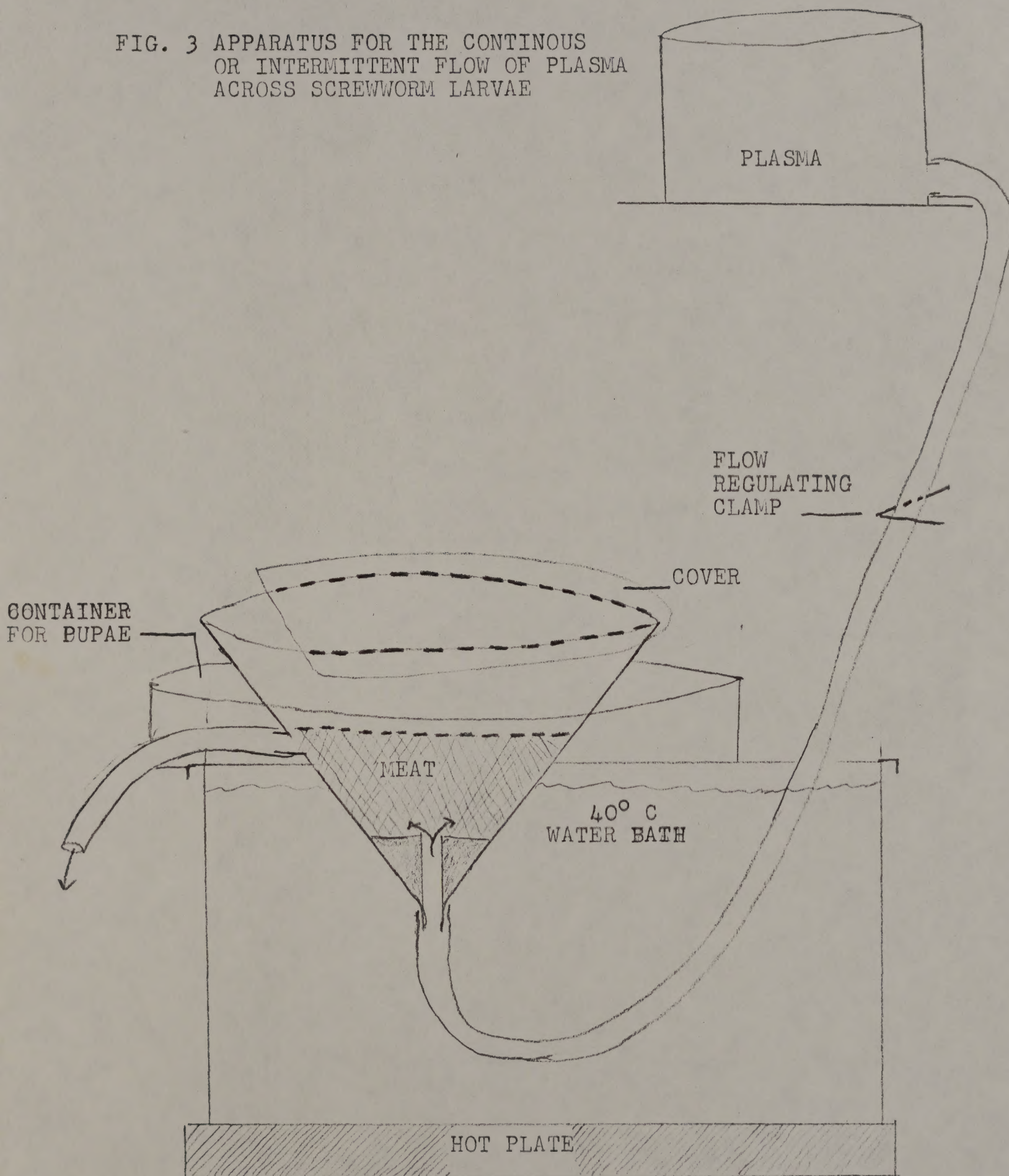


FIG. 3 APPARATUS FOR THE CONTINUOUS
OR INTERMITTENT FLOW OF PLASMA
ACROSS SCREWORM LARVAE



Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

April 9, 1974

Haynie Products, Inc.
5010 York Road
Baltimore, Maryland 21212

Gentlemen:

I have 5 gals. of Atlantic Menhaden Condensed Fish Solubles acidulated with sulfuric acid and 5 gals. of the same material acidulated with phosphoric acid. This material was shipped to Dr. William F. Schwiesow, USDA, ARS, Weslaco, TX. 78596. There is no lot number on the cans or shipping bill. Please see the enclosed bill.

We are planning to try this material as a protein supplement in a screwworm diet. Could you provide me with a proximate analysis of this material? We are mainly interested in the protein and fat levels.

Thank you very much for this information.

Sincerely,

Harold E. Brown
Research Chemist

Enclosure

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

April 24, 1974

Dr. Anthony P. Bimbo
Director of Research
Zapata Haynie Corporation
5010 York Road
Baltimore, Maryland 21212

Dear Dr. Bimbo:

Thank you very much for the data sheets on your acid stabilized Menhaden fish soluble concentrates. We plan to evaluate these as possible protein sources in screwworm diet.

I was particularly interested in the data sheet on your spray dried product. This material probably could fit into our program better than the liquid concentrate since the fat levels may affect the usefulness of the product and could be very easily extracted from the spray dried material. I would appreciate it if you would send me a sample of 25-30 lbs. of the material for inclusion into screwworm diets.

Thank you very much for your consideration.

Sincerely,

Harold E. Brown
Research Chemist

W. F. Schwiesow

May 1, 1974

In Test 4, we deleted the cottage cheese and increased the fish solubles to 4%, this would give us near comparable protein levels as is in their standard hydroponic media. We will blend the fish solubles because of the quantity of material needed to complete the study. Having only 5 gals of each, we will need 2 to 2.25 gal for the separate evaluations, and 2-2.25 of each for Test 4. The total requirements for the study will be ~~4~~4.5 gal.

Fortunately, these fish soluble samples go into solution readily and are at a pH of 4.8 to 5.0; therefore, we see no physical problems incorporating these samples into the media.

We have requested that the Zapata-Haynie Corporation supply us with a sample of their spray dried fish soluble concentrate which we plan to evaluate in future experiments.

Harold E. Brown
Research Chemist

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 1, 1974

Subject: Evaluation of Acid Stabilized Menhaden Fish Soluble Concentrates
in Screwworm Diets

To: W. F. Schwiesow, Assistant Area Director
Sub-Tropical Texas Area

Through: J. Wendell Snow, Research Leader
Screwworm Research Laboratory

We have planned a set of experiments to obtain preliminary information concerning the suitability of using acid stabilized fish solubles in screwworm media. Since the rearing facility at SRL is not equipped or set up to handle "hydro" media, then we have to set it up on the "spur" line in the main plant. This involves using the big trays under in-plant conditions.

Four tests will be set up, in duplicate, and they will include:

Test 1--Standard hydroponic media to be used as the control and to collect additional data on protein utilization by larvae grown on this type of media.

Test 2--Standard hydroponic media plus 2% sulfuric acid stabilized Menhaden fish soluble concentrate.

Test 3--Standard hydroponic media plus 2% phosphoric acid stabilized Menhaden fish soluble concentrate.

Test 4--Hydroponic media less the 2% cottage cheese which is replaced with 4% of acid stabilized Menhaden fish soluble concentrate. Use a 1:1 mixture of sulfuric acid stabilized and phosphoric acid stabilized Menhaden fish soluble concentrates.

According to Jack Graham and Frank Dudley, fish products in the past have been unsuccessfull because of the oil or fat content. The screwworm larvae can't tolerate much fat in the media, an evaluation of 2% fish solubles in the media, along with cottage cheese, would enable us to determine if we can use the fish solubles.

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 1, 1974

Subject: Evaluation of Acid Stabilized Menhaden Fish Soluble Concentrates
in Screwworm Diets

To: W. F. Schuster, Assistant Area Director
Sub-Tropical Texas Area

Through: J. Wendell Snow, Research Leader
Screwworm Research Laboratory

We have planned a set of experiments to obtain preliminary information concerning the suitability of using acid stabilized fish solubles in screwworm media. Since the testing facility at SRI is not equipped or set up to handle "hydro" media, then we have to set it up on the "dry" line in the main plant. This involves using the big trays under in-plant conditions.

Four tests will be set up, in duplicate, and they will include:

Test 1--Standard hydroponic media to be used as the control and to collect additional data on protein utilization by larvae grown on this type of media.

Test 2--Standard hydroponic media plus 2% sulfuric acid stabilized Menhaden fish soluble concentrate.

Test 3--Standard hydroponic media plus 2% phosphoric acid stabilized Menhaden fish soluble concentrate.

Test 4--Hydroponic media less the 2% cottage cheese which is replaced with 4% of acid stabilized Menhaden fish soluble concentrate. Use a 1:1 mixture of sulfuric acid stabilized and phosphoric acid stabilized Menhaden fish soluble concentrates.

According to Jack Graham and Frank Hudley, fish products in the past have been unsuccessful because of the oil or fat content. The screwworm larvae can't tolerate much fat in the media, an evaluation of 2% fish solubles in the media, along with cottage cheese, would enable us to determine if we can use the fish solubles.

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 8, 1974

Dr. John H. Green
Coordinator/Liaison Officer, Pollution Abatement
U. S. Department of Commerce, NOAA, NMFS
College Park Fish Products Tech. Lab. (F273)
Regents Drive, University of Maryland Campus
College Park, Maryland 20740

Dear Dr. Green:

I would like to thank you for the papers on uses of fish solubles in nutrition. I was familiar with some of the "old" work done in the early sixties, and some place in my records, I have a copy of some amino acid analysis on Menhaden fish solubles. This work was done with Dr. J. K. Couch of Texas A&M University.

I have been corresponding with Dr. Anthony P. Bimbo, Director of Research for Zapata Haynie Corporation in Baltimore, and he has forwarded a sample of their spray dried Atlantic Menhaden fish solubles. We plan to try this material as a protein source in screwworm media. The liquid fish soluble concentrates supplied by the Zapata Haynie Corporation were used in an experiment and the results were inconclusive. Some uncontrollable variables in our culture vats caused a high degree of variation in the results. However, the results were encouraging and after trying the spray dried material, we may request additional liquid samples for further evaluations.

I plan to call Dr. Miller, of your facility, in the near future. Maybe he will have some suggestions for the use of this material, along with suggestions of other possible protein sources.

Thank you very much for your interest.

Sincerely,

Harold E. Brown
Research Chemist

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 8, 1974

Dr. Anthony P. Bimbo
Director of Research
Zapata Haynie Corporation
5010 York Road
Baltimore, Maryland 21212

Dear Dr. Bimbo:

Thank you for your letter of April 30 informing me that you were shipping 50 lbs. of your spray dried fish solubles. I have also received a copy of the shipping bill from your Reedville, Virginia, plant.

We have just completed an experiment using your liquid Menhaden fish soluble concentrates, and the results were encouraging but inconclusive. We had some uncontrollable problems in our culture vats which made our results vary excessively. We plan to repeat the experiment when the spray dried material arrives.

Should you have data available concerning cost, etc., of the liquid as well as the spray dried solubles, we would appreciate receiving a set of these figures.

I shall keep you informed of our progress on these experiments.

Sincerely,

Harold E. Brown
Research Chemist

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

May 14, 1974

Dr. R. E. Gingrich
Research Entomologist
U. S. Livestock Insects Laboratory
P. O. Box 232
Kerrville, Texas 78028

Dear Dr. Gingrich:

I am returning the publication by Sedee along with the other information that you loaned me. Many thanks for the use of this material; the Sedee publication has been particularly helpful.

The work is progressing. However it tends to be moving a little slower than I had anticipated. We've spent considerable time trying to establish a chemistry laboratory. The existing facility had pretty well gone to pot and needed revamping.

We've run a couple of protein utilization experiments in the plant and are busy with the analyses. The larvae weights were in an "acceptable" range of 65-70 mg; however, these initial experiments have been designed to determine the degree of protein utilization by larvae grown on the "hydro" media and not at increasing the weights of the larvae. Future experiments will be designed to evaluate various protein sources and altering the protein source in order to increase the weights of the larvae.

Again many thanks for the use of the material and for your hospitality during our visit to your facility. Pass on my regards to Dr. Drummond, Dr. Graham, and Jack Palmer and others.

Sincerely,

Harold E. Brown
Research Chemist

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

November 11, 1974

Subject: Storage of Blood

To: Norvan Meyer
Co-Director
Comision Mexico-Americana Para La Eradicacion
del Gusano Barrenador
Liebnitz No. 20
Mexico 1, D. F.

Enclosed is a letter prepared by our chemist in an effort to answer the question of blood storage.

Apparently it can be stored with less than 6% moisture for an indefinite period.

J. Wendell Snow
Research Leader

Enclosure

Journal of the Royal Society of Medicine
Vol. 30, Part 1
London, 1937

Received 11.11.37

Subject: Storage of Blood

To: Mr. J. H. W. Meyer

Co-Director

Committee on the Storage of Blood

and General Discussion

London, Nov. 10

Dear Sir,

Enclosed is a letter prepared by our committee in an effort to answer
the question of blood storage.

It can be stated with less than 5% confidence that the
letter is correct.

Yours faithfully,

Research Leader

Enclosure

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

November 1, 1974

Subject: Storage Quality of Dried Whole Blood

To: J. Wendell Snow
Research Leader
Screwworm Research Laboratory

In response to your request for me to gather some information concerning the storage of dried whole blood, I have contacted several persons and/or companies. The initial inquiries on dried whole blood storage were directed to Mr. A. J. Graham. They had kept no absolute records on blood storage but had used blood that had been on hand as long as 1 year, he thought, without any obvious problems. Other dry products used in the hydroponic media have posed insect problems during storage, but none were encountered with the blood.

I called Mr. Ben Smith of Rath Packing Co., Waterloo, Iowa, and he informed me that they had stored dry blood for as long as four years without any apparent damage. He stated that several criteria should be met before trying to store blood. The blood should be dried to around 5% residual moisture, and at no time should the moisture content be allowed to exceed 8%. When the moisture content exceeds 8%, the product becomes clumpy and decomposition occurs. Storage should be in heavy duty, double-walled paper bags with a polyethylene liner. Care should be exercised to avoid mechanical damage to the bags. Mr. Smith stated that they had no insect problem in their stored dry blood and no decomposition occurred during storage. He said that rodent infestations in the storage area could be a problem.

I talked, by phone, with Mr. Carl Wild of Inland Molasses Co., Dubuque, Iowa, about storage problems of dried whole blood. He had many of the same opinions as Mr. Smith of Rath in that he would anticipate no problems as long as the dried blood remained dry. He used the 8% residual moisture figure as the upper limit for stored blood. He stated that his company had no experience in storage of blood and maintained only a small storage facility. He also stated that they were mainly bulk shippers of drip blood, and the only experience they had in bagging was shipped to Mission. These were not bagged with a polyethylene liner in the bag.

He questioned the wisdom of obtaining large quantities of blood requiring long-term storage, and as I didn't know the background of my request, I did not discuss this problem with him.

I discussed the problem of storage of dry blood with Dr. J. M. Prescott, Dean, School of Science, Texas A&M University. Dr. Prescott is a Protein Chemist and has had considerable experience in related fields, but not in storage of dry blood. However, Dr. Prescott said that he would not anticipate any problems in storage for periods of 1 year or even longer as long as the products are kept out of the direct sunlight and dry. He stated that the product should be dried to around 5-6% moisture and maintained at this level during storage.

I talked with Bill Sudlow, who was here from the Mexican-American Program, and Bill stated that he would not expect and had not experienced any degradation of stored dry blood. He said that he had experienced deterioration in other dry products used in the media, from both insect damage and spoilage, but had not experienced this with the dry blood. Again, he thought the success of storage depended on adequate packaging of blood in order to keep it dry, not allowing the blood to absorb moisture from the air.

My experience with dried whole blood since I've been with the Screwworm Program has been that the drier bloods mix better. The bloods used here range in residual moisture from around 6% to 11%. The 11% moisture blood (Canadian blood) tended to give us the most problems. Therefore, we may assume that the elevated moisture content would also lend itself to quicker degradation in storage. We do know, in other products such as food, enzyme preparations, antibiotic preparations, etc., that normal drying to around 10% moisture is often not good enough for prolonged storage, but the reduction of moisture to around 4% by freeze drying will allow the product to be stored at ambient temperature with a minimal loss of nutrient value and biological activity. Another plus for the storage of dried blood is that the lipid levels are less than 1% of the product; therefore, minimal degradation will occur due to the autooxidation of the lipids in storage.

Should other information be needed, please inform me.

Harold E. Brown
Research Chemist

Screwworm Research Laboratory
P. O. Box 986
Mission, Texas 78572

November 18, 1974

Dr. Anthony P. Bimbo
Director of Research
Zapata Haynie Corp.
5010 York Road
Baltimore, Maryland 21212

Dear Dr. Bimbo:

Thank you for your letter of November 5, 1974, inquiring about the results of our experiments on rearing screwworm larvae using your fish products as a protein source.

As I had reported to you in earlier phone conversations, your samples of acid stabilized Menhaden fish solubles, both liquid and spray dried, did not support growth of the larvae. In fact, growth was somewhat retarded when these products were added to the rearing media. We initially thought that the lipid levels were responsible for the reduced growth; however, we tried the spray dried, low fat solubles and the response was similar to that obtained with the fish solubles with normal lipid levels. These results have been obtained in experiments where we had tried to replace dried cottage cheese in the media by addition and deletion techniques. There have been reports that the histamine levels in fish products inhibit growth of the larvae.

We have received the Gulf Menhaden Fish Meal and the Experimental Peptone, RS-7; however, I have not had time to evaluate these products. About the time they arrived, I had a flare-up of some type of stomach condition, spent some time in the hospital and more time convalescing, and have not had time to get these tests completed. I plan to start on these experiments next week and will inform you of the results.

In the past, we have performed these experiments in the production plant, under plant conditions. Often the conditions on the rearing floor are difficult to control and tend to introduce inconsistent variables in the experimentations. We plan to perform the remainder of the fish product experiments in the plant, but are planning to establish a facility for conducting these experiments with liquid media. This will

Research Laboratory
P. O. Box 300
Mission, Texas 75152

November 12, 1971

Dr. Stanley P. Hing
Director of Research
Japan Marine Corp.
501 York Road
Beltsville, Maryland 21112

Dear Dr. Hing:

Thank you for your letter of November 5, 1971, regarding the
results of our experiments on feeding behavior of the
fish products as a protein source.

As I had reported to you in earlier phone conversations, your
of and established methods with which to feed fish and other
did not support growth of the larvae. In fact, growth was somewhat
reduced when these products were added to the feeding water. We
initially thought that the lipid levels were responsible for the
reduced growth. However, we tried the spray dried, low fat solution
and the response was similar to that obtained with the fish solution
with normal lipid levels. These results have been obtained in experi-
ments where we had tried to replace dried codfish oil in the water
by addition and isolation techniques. There have been reports that the
lipid levels in fish products inhibit growth of the larvae.

We have received the full formulation fish meal and the experimental
results. As to the growth, I have not had time to evaluate these products
since the time they arrived. I had a flu and was out of the office.
Additionally, some time in the hospital and some time conducting
and have not had time to set these tests completed. I plan to start
on the experiments next week and will inform you of the results.

In the past, we have performed these experiments in the experimental
under strict conditions. Since the conditions on the experimental floor are
difficult to control and hard to reproduce consistently, we have
the experimental results. We plan to perform the remainder of the fish
product experiments in the fish tank and are planning to establish a
facility for conducting these experiments with fish tanks. This will

Anthony P. Bimbo

November 18, 1974

allow us to control the conditions more closely and will allow possibly for a better utilization of experimental materials as we will scale down the rearing vats thus reducing the nutrient requirements on the vats.

Thank you for the offer of additional materials; as we need them we will be contacting you. Again, when we have completed the evaluations of the fish products, I'll be forwarding a report to you.

Thank you very much for your interest.

Sincerely,

Harold E. Brown
Research Chemist

REFERENCE SLIP

3/14/73

TO

R. C. Bushland

16 MAR 1973

☐ ACTION☐ NOTE AND RETURN☐ APPROVAL☐ PER PHONE CALL☐ AS REQUESTED☐ RECOMMENDATION☐ FOR COMMENT☐ REPLY FOR SIGNATURE OF☒ FOR INFORMATION☐ RETURNED☐ INITIALS☐ SEE ME☐ NOTE AND FILE☐ YOUR SIGNATURE

REMARKS

FROM

Edgar A Taylor

Bushland
ET

16 MAR 1973

FEB 27 1973

Beltsville, Maryland 20705

February 22, 1973

Subject: Request for Assistance on Screwworm Nutrition

To: W. M. Bruce, Area Director, Tifton, Georgia

Two weeks ago APHIS and ARS scientists met in Washington, D. C., to review the screwworm program. As you know after 9 trouble-free years the program was a disappointment in the last season. Losses to the cattle industry were about \$40 million in 1972.

Although corrective actions have been taken, there is strong evidence that the released sterile males are performing poorly. Drs. E. F. Knipping and R. C. Bushland believe that the artificial diet is nutritionally inadequate. They strongly recommended that Dr. Ray F. Moore, Florence, South Carolina, spend a number of days at the mass rearing facility at McAllen and attempt to analyze the difficulty.

I spoke to Dr. M C Cox by telephone, and he suggested that I clear this matter with you. In addition Dr. Cox stated that he is willing to discuss practical problems in sending Dr. Moore to McAllen with you.

If Dr. Moore can make a contribution, it should be made in advance of the next fly season.

Waldemar Klassen
Waldemar Klassen
Staff Scientist
Pest Management

cc:
L. S. Henderson
E. A. Taylor ✓

Bushland
SKT

FEB 27 1973

Beltsville, Maryland 20705

February 22, 1973

Subject: Request for Assistance on Screwworm Nutrition

To: J. Rex Johnston, Area Director, College Station, Texas

Two weeks ago APHIS and ARS scientists met in Washington, D. C., to review the screwworm program. As you know, after 9 trouble-free years the program was a disappointment in the last season. Losses to the cattle industry were about \$40 million in 1972.

Although corrective actions have been taken, there is strong evidence that the released sterile males are performing poorly. Drs. E. F. Knippling and R. C. Bushland believe that the artificial diet is nutritionally inadequate. They strongly recommend that Dr. R. Gingrich, Kerrville, Texas, spend a number of days at the mass rearing facility at McAllen and attempt to analyze the difficulty.

I spoke to Dr. H C Cox by telephone, and he suggested that I clear this matter with you. In addition Dr. Cox stated that he is willing to discuss with you practical problems in sending Dr. Gingrich to McAllen.

If Dr. Gingrich can make a contribution, it should be made in advance of the next fly season.

Waldemar Klassen

Waldemar Klassen
Staff Scientist
Pest Management

cc:

L. S. Henderson
E. A. Taylor✓

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
SOUTHERN REGION
701 LOYOLA AVENUE
P. O. BOX 53326
NEW ORLEANS, LOUISIANA 70153

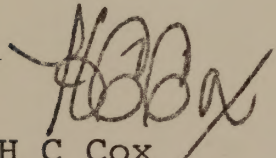
Mr. Bushland
Missing Sep
BS
14 MAR 1973

March 12, 1973

Subject: Assistance on Screwworm Nutrition Research

To: The Record

Subsequent to a recommendation by Dr. Waldemar Klassen that Drs. R. F. Moore and Richard Gingrich visit Mission to see if they could provide assistance on a problem in screwworm rearing which is apparently associated with nutrition, I talked by telephone with Mr. Bruce, Dr. Bushland, and Dr. Moore. Mr. Bruce was concerned that Dr. Bushland might not be aware of the proposed visit by Drs. Moore and Gingrich. However, Dr. Bushland assured me that he would welcome their aid. I asked Dr. Moore to contact Dr. Bushland and work out an appropriate time when he and Dr. Gingrich could visit the Mission Laboratory. Dr. Moore expressed concern about a lack of travel funds, particularly since the trip would be in support of a project other than his own. I asked Dr. Moore to perform the travel and send us a voucher or some statement of costs incurred, assuring him that he would be reimbursed for funds expended.


H C Cox
Associate Deputy Administrator

cc:

N. S. Bridges
J. R. Johnston, Bushland, Tex.
W. M. Bruce, Tifton, Ga.
R. O. Drummond, Kerrville, Tex.
H. M. Taft, Florence, S. C.
W. Klassen, NPS, Beltsville, Md.
R. C. Bushland, Mission, Texas
E. A. Taylor, Weslaco, Texas

